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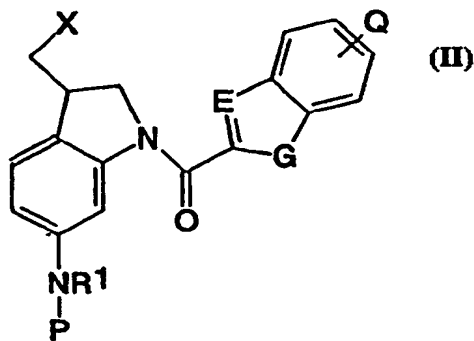
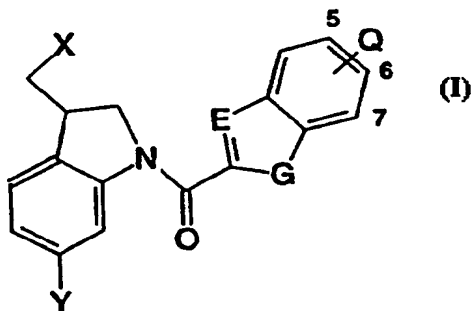
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(54) Title: CYCLOPROPYLINDOLES AND THEIR SECO PRECURSORS, AND THEIR USE AS PRODRUGS



(57) Abstract

The present invention provides compounds of formula (I) and (II) which may be used as anticancer drugs.

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CYCLOPROPYLINDOLES AND THEIR SECO PRECURSORS, AND THEIR USE AS PRODRUGS

The present invention relates to novel amino analogues of the general class of cyclopropylindoles and their seco precursors, and is particularly concerned with the use of these compounds as prodrugs for antibody-directed enzyme-prodrug therapy (ADEPT) and gene-directed enzyme-prodrug therapy (GDEPT) for cancer.

Background to the invention.

The use of prodrugs represents a clinically very valuable concept in cancer therapy since, particularly where the prodrug is to be converted to an anti-tumour agent under the influence of an enzyme that is linkable to a monoclonal antibody that will bind to a tumour associated antigen, the combination of such a prodrug with such an enzyme monoclonal/antibody conjugate represents a very powerful clinical agent. This approach to cancer therapy, often referred to as "antibody directed enzyme/prodrug therapy" (ADEPT) is disclosed in WO88/07378.

A further therapeutic approach termed "virus-directed enzyme prodrug therapy" (VDEPT) has been proposed as a method for treating tumour cells in patients using prodrugs. Tumour cells are targeted with a viral vector carrying a gene encoding an enzyme capable of activating a prodrug. The gene may be transcriptionally regulated by tissue specific promoter or enhancer sequences. The viral vector enters tumour cells and expresses the enzyme, in order that a prodrug is converted to an active drug within the tumour cells (Huber et al, Proc. Natl. Acad. Sci. USA (1991) 88, 8039). Alternatively, non-viral methods for the delivery of genes have been used. Such methods include calcium phosphate co-precipitation, microinjection, liposomes, direct DNA uptake, and receptor-mediated DNA transfer. These are reviewed in Morgan & French, Annu. Rev. Biochem., 1993, 62;191. The term "GDEPT" (gene-directed enzyme prodrug therapy) is used to include both viral and non-viral delivery systems.

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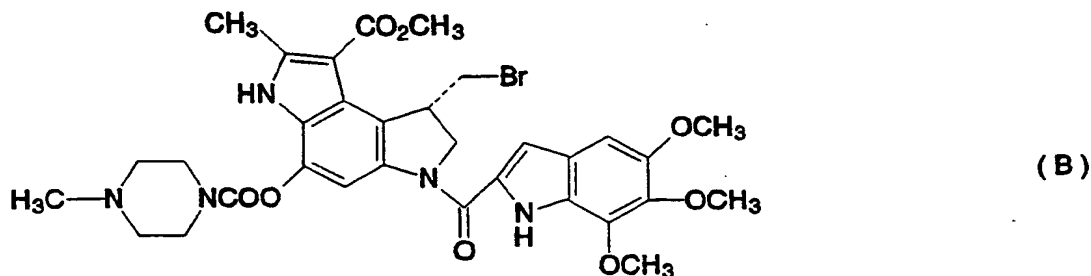
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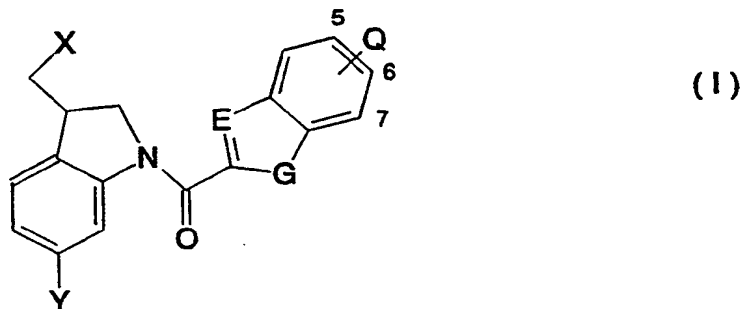
A related compound is KW-2189 (E. Kobayashi et al, Cancer Res., 54, 1994, 2404-2410) which has the structure B:



Both carzelesin and KW-2189 show anticancer activity against a range of human tumours and are in clinical trial. Further analogues of a similar type are disclosed in WO88/04659 and WO91/16324.

Disclosure of the Invention.

In one aspect, the present invention relates to the new class of substituted seco indolines, represented by formula (I):



wherein:

X is halogen or OSO_2R , where R represents H or lower straight or branched alkyl (up to five carbon atoms) optionally substituted with from 1 to 4 hydroxyl, acid (COOH) or amino groups which amino groups are optionally substituted by one or two lower alkyl groups;

Y is NH_2 , NO_2 , NHOH , NHR , NRR , N(O)RR , NROH , SR or SSR , where R is defined as above, but that in the case where Y is SSR , then

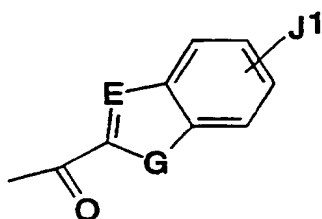
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R can also be another moiety of formula (I) (i.e., a symmetrical disulfide);

E is -N= or -CH=;

G is O, S or NH; and

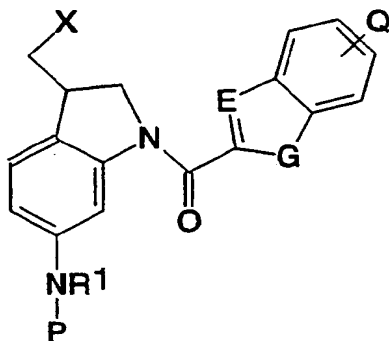
- 5 Q is from one to three of H, OR, NRR, CONHR, NHCOR or NHCONHR at any one of positions 5 to 7 where R is defined as above (which may be the same or different when Q is two or three), a group of formula (Ia):



(Ia)

- 10 where E and G are as defined above, J¹ is up to three groups, H, OR, NRR, CONHR or NHCOR (which may be the same or different when J¹ is two or three) where R is as defined above, or is a group of the formula -CONHJ², -NHJ² or -OJ² where J² is a group -(CH₂)_mHt where m is an integer from 1 to 4 and Ht is a carbon or
 15 heterocyclic ring containing 5 or 6 atoms, one or two of which may be oxygen, sulphur or nitrogen (the remainder being carbon);
 or a physiologically functional derivative thereof.

In a second aspect, the present invention relates to the class of compounds represented by formula (II):

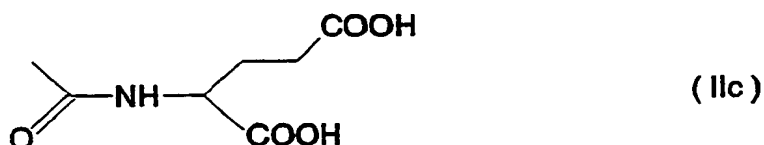
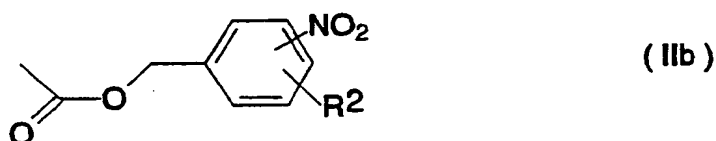
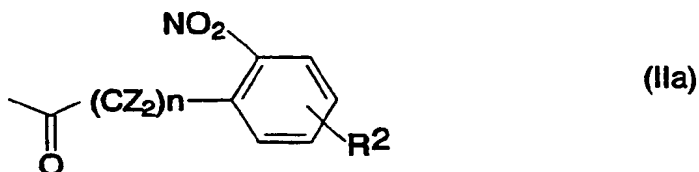


(II)

- 20 wherein:

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X, Q, E and G are as defined above for formula (I), R^1 is a group R as defined above and P is selected from the structures of formulae (IIa, IIb or IIc):



wherein:

- 5 Z is H or Me;
 n is 1 or 2; and
 R^2 is a group R, CONHR, NHCOR, OR or SO_2R , where R is as defined above;
 or a physiologically functional derivative thereof.

10 It is recognised that compounds of formulae (I), (II) and (IIa-c) may exist in different enantiomeric or diastereomeric forms. In such cases it is to be understood that the above formulae represent any possible enantiomeric or diastereomeric form or a mixture thereof.

15 A halogen group means a fluoro, chloro, bromo or iodo group. A chloro group is preferred. Preferred compounds of formulae (I)

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and (II) include those in which X represents Cl. It is preferred that Q represents H or 5,6,7-triOMe and P represents formula IIb, where R² represents H or CONHR, where R is defined as above. It is preferred that where Q represents a group of the formula (Ia) it is attached at the 5- or 6-positions.

Preferred values of Q include:

- CONHCH₂CH₂Nmorpholide,
- CONHCH(CH₂OH)₂,
- CONHCH₂CH₂(2-pyridyl),
- 10 -CONHCH₂CH₂N(Me)₂,
- CONHCH₂CH₂CH₃,
- CONHCH₂CH₂COOH,
- NHCOCH₂CH₂N(Me)₂, and
- NHCOCH₂CH₂COOH.

15 When J² is a group -(CH₂)_mHt m is preferably 2 and Ht is preferably a 5 or 6 membered carbon ring or a 5 or 6 membered ring containing one oxygen and/or one nitrogen which are not adjacent when both are present, and is most preferably Nmorpholide (i.e. a morpholino group attached at the nitrogen) or pyridyl.

20 These groups are desirably linked to the 5 or 6 position of the formula (I) or (II) nucleus.

The group R¹ in formula (II) is preferably hydrogen or methyl.

It is preferred that when P is a group (IIb) the nitro group is in the 4- (para) or 2-position. The 4-position is preferred.

25 In another aspect, the present invention relates to the use of the compounds of formulae (I) and (II) as anticancer drugs. The compounds may be used for the selective killing of oxic and hypoxic tumour cells in methods of treatment of cancers for example leukaemias, and particularly solid cancers including
30 breast, bowel and lung tumours, including small cell lung carcinoma.

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In a further aspect, the present invention relates to the use of the compounds of formula (II), in conjunction the nitroreductase or carboxypeptidase enzymes (for example isolated from E. coli) in methods of ADEPT or GDEPT therapy. Compounds of the formula (I) in which Y is NO₂ or N(O)RR may also be used in conjunction with nitroreductase.

The invention also provides pharmaceutical compositions comprising a compound of the formula (I) or the formula (II) together with a pharmaceutically acceptable carrier or diluent.

Detailed description of the invention.

A. Synthesis of compounds of the formula (I).

The compounds of formula (I) can be prepared by the processes outlined for specific examples in Schemes 1 and 2. In Scheme 1, 4-chloro-3-nitrobenzoic acid is converted to its t-butyl ester and condensed with the sodium salt of dimethyl malonate. The t-butyl ester is cleaved and the resulting acid is subjected to Curtius rearrangement to give the Cbz-protected aniline. Reduction of the malonate with diisobutylaluminium hydride yields the corresponding diol. The nitro group is then selectively reduced by hydrogenation over platinum oxide. Protection of the resulting amine with di-t-butylcarbonate, followed by cyclisation with diethylazodicarboxylate forms the indoline ring. The hydroxymethyl group at the 3-position is converted to a sulphonate by reaction with RSO₂Cl (when X is SO₂R; in the example of scheme 1, R is CH₃), and the Boc protecting group cleaved by HCl. The resulting unstable amine hydrochloride is immediately coupled with the appropriate carboxylic acid (in the example of Scheme 1, 5,6,7-trimethoxyindole-2-carboxylic acid is used, illustrating the case where E is -CH=, G is NH and Q is 5,6,7-trimethoxy). The Cbz protecting group is removed by hydrogenolysis. The mesylate is displaced by the group X (in the case of Scheme 1 by chloride) by reaction with lithium halide (when X is halogen).

In Scheme 2, the t-butyl ester of 2-chloro-5-nitrobenzoic acid is condensed with dimethyl malonate, and converted to the free acid using the same procedure as in Scheme 1. Reaction with

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diphenylphosphoryl azide in the presence of triethylamine leads directly to the 2-indolone, via intramolecular trapping of the intermediate isocyanate. Reduction with borane gives the corresponding indoline, and the indoline nitrogen is protected with the Boc group. Treatment of the diester with sodium methoxide results in decarboxymethylation to give the monoester derivative, which is reduced with diisobutylaluminium hydride. The resulting alcohol is treated with RSO_2Cl (when X is OSO_2R) and then displaced with lithium halide (when X is halogen). In Scheme 2, treatment with lithium chloride is shown to provide the chloro compound, which is coupled with the appropriate carboxylic acid (in the example of Scheme 1, 5,6,7-trimethoxyindole-2-carboxylic acid is used, illustrating the case where E is $-\text{CH}=\text{}$, G is NH and Q is 5,6,7-trimethoxy).

Reduction of the 6-nitro group by hydrogenation over platinum oxide then gives the compound of the formula I where Y is NH_2 .

B. Synthesis of compounds of the formula (II).

The compounds of formula (IIa) and (IIb) may be prepared by reaction of compounds of the formula (I) with a reactive derivative of the group P, for example the acid chloride derivative of (IIa) or the chloroformate derivative of (IIb). Such reactive intermediates may be made from the carboxylic acid derivatives of (IIa) or alcohol derivatives of (IIb). Such reactive derivatives may be made from carboxylic acids of the formula P-H (where P is as defined above). The carboxylic acids and alcohols may be made by chemistry known per se. Some compounds are commercially available. Scheme 1 illustrates this with 4-nitrobenzylchloroformate.

Compounds of the formula (IIc) may be made by coupling glutamic acid derivatives, such as isocyanates, with compounds of the formula (II) in which P is hydrogen. The carboxy groups of glutamic acid may be protected by esterification with C_{1-6} alkyl protecting groups, the *t*-butyl ester groups being preferred. Where such ester groups are used, they may be removed after reaction of the glutamic acid derivative with the compound of

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formula (I) by hydrolysis. In some cases this may result in racemisation.

Reference may be made to, for example, WO88/07378 and WO91/03460 for appropriate reaction conditions for production of glutamic acid derivatives of compounds of formula (II).

Reference may also be made to the synthetic routes disclosed in WO88/04659 and WO91/16324 especially those in which Q is a group of the formula (Ia). Analogous routes may be used to make compound of the present invention.

C. GDEPT.

C(i) - Vector systems.

In general, the vector for use in GDEPT therapies may be any suitable DNA or RNA vector.

Suitable viral vectors include those which are based upon a retrovirus. Such vectors are widely available in the art. Huber et al (ibid) report the use of amphotropic retroviruses for the transformation of hepatoma, breast, colon or skin cells. Culver et al (Science (1992) 256; 1550-1552) also describe the use of retroviral vectors in GDEPT. Such vectors or vectors derived from them may also be used. Other retroviruses may also be used to make vectors suitable for use in the present invention. Such retroviruses include rous sarcoma virus (RSV).

Englehardt et al (Nature Genetics (1993) 4; 27-34) describe the use of adenovirus based vectors in the delivery of the cystic fibrosis transmembrane conductance product (CFTR) into cells, and such adenovirus based vectors may also be used. Vectors utilising adenovirus promoter and other control sequences may be of use in delivering a system according to the invention to cells in the lung, and hence useful in treating lung tumours.

Other vector systems including vectors based on the Molony murine leukaemia virus are known (Ram, Z et al, Cancer Research (1993) 53; 83-88; Dalton & Treisman, Cell (1992) 68; 597-612). These vectors contain the Murine Leukaemia virus (MLV) enhancer cloned

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upstream at a β -globin minimal promoter. The β -globin 5' untranslated region up to the initiation ATG is supplied to direct efficient translation of the enzyme.

5 Suitable promoters which may be used in vectors described above, include MLV, CMV, RSV and adenovirus promoters. Preferred adenovirus promoters are the adenovirus early gene promoters. Strong mammalian promoters may also be suitable. An example of such a promoter is the EF-1 α promoter which may be obtained by reference to Mizushima and Nagata ((1990), Nucl. Acids Res. 18;
10 5322). Variants of such promoters retaining substantially similar transcriptional activities may also be used.

C(ii) - Nitroreductase.

Compounds of the formula (II) in which P is a group (IIa) or (IIb) can be activated by removal of the group P by nitroreductase.

15 Preferably, the enzyme is a non-mammalian nitroreductase enzyme, such as a bacterial nitroreductase. An E.coli nitroreductase as disclosed in WO93/08288 is particularly preferred. The enzyme may be modified by standard recombinant DNA techniques, e.g. by cloning the enzyme, determining its gene sequence and altering the
20 gene sequence by methods such as truncation, substitution, deletion or insertion of sequences for example by site-directed mutagenesis. Reference may be made to "Molecular Cloning" by Sambrook et al (1989, Cold Spring Harbor) for discussion of standard recombinant DNA techniques. The modification made may
25 be any which still leaves the enzyme with the ability to reduce the nitro group of the protecting group P in formula II or the nitro or amine N-oxide groups when these are represented by Y in formula I but alters other properties of the enzyme, for example its rate of reaction or selectivity.

30 In addition, small truncations in the N- and/or C-terminal sequence may occur as a result of the manipulations required to produce a vector in which a nucleic acid sequence encoding the enzyme is linked to the various other vector sequences.

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C(iii) Carboxypeptidase

Compounds of the formula (II) in which P is a group (IIc) can be activated by removal of the group P by a carboxypeptidase enzyme.

The enzyme is preferably a bacterial carboxypeptidase, especially carboxypeptidase CPG2 or Pseudomonas γ -glutamylhydrolase EC3.4.22.12 (Levy CC & Goldman P J. Biol. Chem. 242; p2933 (1967)).

Carboxypeptidase G2 (CPG2) is disclosed in WO88/07378. Although native CPG2 is preferred, alterations to its sequence which are amino acid substitutions, deletions or insertions (eg. of about 1, 2, 3, 4, 5, 10 or 20 residues in each case) are also possible. In any event, the alteration will be such that the enzyme retains its ability to convert a prodrug to an active drug at substantially the same rate as the native enzyme. In this context, "substantially the same rate" will desirably be within 1 order of magnitude, and preferably from about 50-fold e.g. about 2-fold less to 2, 5 or 10 fold more.

In addition to specific changes the enzyme may otherwise be altered by truncation, substitution, deletion or insertion as long as the activity of the enzyme is substantially unchanged as defined above. For example, small truncations in the N- and/or C-terminal sequence may occur as a result of the manipulations required to produce a vector in which a nucleic acid sequence encoding the enzyme is linked to a suitable promoter.

D. ADEPT.

For applications in ADEPT systems, an antibody directed against a tumour specific marker is linked to the nitroreductase or carboxypeptidase enzyme, which may be modified as described above. The antibody may be monoclonal or polyclonal. For the purposes of the present invention, the term "antibody", unless specified to the contrary, includes fragments of whole antibodies which retain their binding activity for a tumour target antigen. Such fragments include Fv, F(ab') and F(ab')₂ fragments, as well as single chain antibodies. Furthermore, the antibodies and

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fragments thereof may be humanised antibodies, eg. as described in EP-A-239400.

The antibodies may be produced by conventional hybridoma techniques or, in the case of modified antibodies or fragments, by recombinant DNA technology, eg by the expression in a suitable host vector of a DNA construct encoding the modified antibody or fragment operably linked to a promoter. Suitable host cells include bacterial (eg. *E.coli*), yeast, insect and mammalian. When the antibody is produced by such recombinant techniques the enzyme may be produced by linking a nucleic acid sequence encoding the enzyme (optionally modified as described above) to the 3' or 5' end of the sequence of the construct encoding the antibody or fragment thereof.

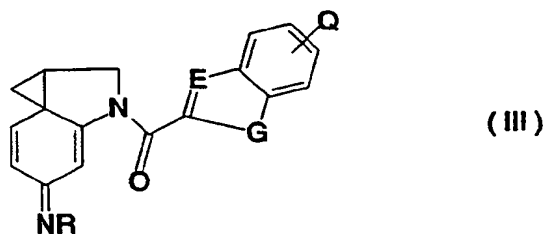
E. Physiologically functional derivatives.

Physiologically functional derivatives of prodrugs include salts, amides and esters. Esters include carboxylic acid esters in which the non-carbonyl moiety of the ester grouping is selected from straight or branched chain C₁₋₆alkyl, (methyl, n-propyl, n-butyl or t-butyl); or C₃₋₆cyclic alkyl (e.g. cyclohexyl). Salts include physiologically acceptable base salts, eg derived from an appropriate base, such as alkali metal (e.g. sodium), alkaline earth metal (e.g. magnesium) salts, ammonium and NR₄. (wherein R is C₁₋₄ alkyl) salts. Other salts include acid addition salts, including the hydrochloride and acetate salts. Amides include non-substituted and mono- and di-substituted derivatives. Such derivatives may be prepared by techniques known per se in the art of pharmacy.

Other physiologically functional derivatives may occur by formation of such derivatives in the body after administration of the compounds of the invention.

For example, many of the compounds of formula (I) and (II), particularly those where Y is NH₂ or NHR may be cytotoxic via a cyclopropylimine structure of formula (III):

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where R, E, G and Q are as defined above. The imine group (=NR) may also arise when cleavage of the group P occurs in compounds of the formula (II).

F. Applications of the invention.

5 The compounds of the invention can be used in a method of treatment of the human or animal body. Such treatment includes a method of treating the growth of neoplastic cells in a patient with neoplastic disease which comprises administering to a patient in need of treatment compounds of formula (I) of the invention, or compounds of formula (II) of the invention as part of an ADEPT
10 or GDEPT therapy system. Neoplastic diseases include leukaemia and solid tumours such as breast, bowel and lung tumours including small cell lung carcinoma.

15 It will be understood that where treatment of tumours is concerned, treatment includes any measure taken by the physician to alleviate the effect of the tumour on a patient. Thus, although complete remission of the tumour is a desirable goal, effective treatment will also include any measures capable of achieving partial remission of the tumour as well as a slowing
20 down in the rate of growth of a tumour including metastases. Such measures can be effective in prolonging and/or enhancing the quality of life and relieving the symptoms of the disease.

F(i): Compounds of the formula (I).

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Compounds of the formula (I) of the present invention may be used in a method of treatment of neoplastic disease in a patient, which method comprises administering to a patient in need of treatment an effective amount of a compound of formula (I). The compound
5 may be administered in the form of a pharmaceutical composition.

While the exact dose of the compound will be at the discretion of the physician, taking account of the condition and needs of the patient, typical doses will be in the range of from about 0.1 to 200 mg/Kg, preferably about from 10 to 100 mg/Kg per patient per
10 day.

F(ii): ADEPT therapy.

The antibody/enzyme conjugate for ADEPT can be administered simultaneously but it is often found preferable, in clinical practice, to administer the enzyme/agent conjugate before the
15 prodrug, e.g. up to 72 hours or even 1 week before, in order to give the enzyme/agent conjugate an opportunity to localise in the region of the tumour target. By operating in this way, when the prodrug is administered, conversion of the prodrug to the cytotoxic agent tends to be confined to the regions where the
20 enzyme/agent conjugate is localised, i.e. the region of the target tumour the premature release of the compound of formula (II) is minimised.

In ADEPT the degree of localisation of the enzyme/agent conjugate (in terms of the ratio of localized to freely circulating active
25 conjugate) can be further enhanced using the clearance and/or inactivation systems described in WO89/10140. This involves, usually following administration of the conjugate and before administration of the prodrug, the administration of a component (a "second component") which is able to bind to the such part of
30 the conjugate so as to inactivate the enzyme and/or accelerate the clearance of the conjugate from the blood. Such a component may include an antibody to the enzyme component of the system which is capable of inactivating the enzyme.

The second component may be linked to a macromolecule such as
35 dextran, a liposome, albumin, macroglobulin or a blood group O

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erythrocyte so that the second component is restrained from leaving the vascular compartment. In addition or as an alternative, the second component may include a sufficient number of covalently bound galactose residues, or residues of other sugars such as lactose or mannose, so that it can bind the conjugate in plasma but be removed together with the conjugate from plasma by receptors for galactose or other sugars in the liver. The second component should be administered and designed for use such that it will not, to any appreciable extent, enter the extravascular space of the tumour where it could inactivate localised conjugate prior to and during administration of the prodrug.

In ADEPT systems, the dose of the prodrug and conjugate will ultimately be at the discretion of the physician, who will take into account such factors as the age, weight and condition of the patient. Suitable doses of prodrug and conjugate are given in Bagshawe et al. Antibody, Immunoconjugates, and Radiopharmaceuticals (1991), 4, 915-922. A suitable dose of conjugate may be from 500 to 200,000 enzyme units/m² (e.g. 20,000 enzyme units/m²) and a suitable dose of prodrug may be from about 0.1 to 200 mg/Kg, preferably about from 10 to 100 mg/Kg per patient per day.

In order to secure maximum concentration of the conjugate at the site of desired treatment, it is normally desirable to space apart administration of the two components by at least 4 hours. The exact regime will be influenced by various factors including the nature of the tumour to be targeted and the nature of the prodrug, but usually there will be an adequate concentration of the conjugate at the site of desired treatment within 48 hours.

The ADEPT system when used with nitroreductase also preferably comprises a suitable cofactor for the enzyme. Suitable cofactors include a riboside or ribotide of nicotinic acid or nicotinamide.

The antibody/enzyme conjugate may be administered by any suitable route usually used in ADEPT therapy. This includes parenteral

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administration of the antibody in a manner and in formulations similar to that described in section F(iv) below.

F(iii): GDEPT therapy.

For use of the vectors in therapy, the vectors will usually be packaged into viral particles and the particles delivered to the site of the tumour, as described in for example Ram et al (ibid). The viral particles may be modified to include an antibody, fragment thereof (including a single chain) or tumour-directed ligand to enhance targeting of the tumour. Alternatively the vectors may be packaged into liposomes. The liposomes may be targeted to a particular tumour. This can be achieved by attaching a tumour-directed antibody to the liposome. Viral particles may also be incorporated into liposomes. The particles may be delivered to the tumour by any suitable means at the disposal of the physician. Preferably, the viral particles will be capable of selectively infecting the tumour cells. By "selectively infecting" it is meant that the viral particles will primarily infect tumour cells and that the proportion of non-tumour cells infected is such that the damage to non-tumour cells by administration of a prodrug will be acceptably low, given the nature of the disease being treated. Ultimately, this will be determined by the physician.

One suitable route of administration is by injection of the particles in a sterile solution. Viruses, for example isolated from packaging cell lines may also be administered by regional perfusion or direct intratumoral direction, or direct injection into a body cavity (intracavitary administration), for example by intra-peritoneum injection.

The exact dosage regime for GDEPT will, of course, need to be determined by individual clinicians for individual patients and this, in turn, will be controlled by the exact nature of the prodrug and the cytotoxic agent to be released from the prodrug but some general guidance can be given. Chemotherapy of this type will normally involve parenteral administration of modified virus and administration by the intravenous route is frequently found to be the most practical.

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In GDEPT systems the amount of virus or other vector delivered will be such as to provide a similar cellular concentration of enzyme as in the ADEPT system mentioned above. Typically, the vector will be administered to the patient and then the uptake of the vector by transfected or infected (in the case of viral vectors) cells monitored, for example by recovery and analysis of a biopsy sample of targeted tissue. This may be determined by clinical trials which involve administering a range of trial doses to a patient and measuring the degree of infection or transfection of a target cell or tumour. The amount of prodrug required will be similar to or greater than that for ADEPT systems.

In using a GDEPT system the prodrug will usually be administered following administration of the vector encoding an enzyme. Suitable doses of prodrug are from about 0.1 to 200 mg/Kg, preferably about from 10 to 100 mg/Kg per patient per day.

F(iv): Administration of drug or prodrug.

While it is possible for the compounds of formula (I) or the prodrugs of formula (II) to be administered alone it is preferable to present them as pharmaceutical formulations. The formulations comprise the compounds, together with one or more acceptable carriers thereof and optionally other therapeutic ingredients. The carrier or carriers must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipients thereof, for example, liposomes. Suitable liposomes include, for example, those comprising the positively charged lipid (N[1-(2,3-dioleyloxy)propyl]-N,N,N-triethylammonium (DOTMA), those comprising dioleoylphosphatidylethanolamine (DOPE), and those comprising 3β [N-(n',N'-dimethylaminoethane)-carbamoyl]cholesterol (DC-Chol).

Formulations suitable for parenteral or intramuscular administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats, bactericidal antibiotics and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending

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agents and thickening agents, and liposomes or other microparticulate systems which are designed to target the compound to blood components or one or more organs. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water, for injections, immediately prior to use. Injection solutions and suspensions may be prepared extemporaneously from sterile powders, granules and tablets of the kind previously described.

It should be understood that in addition to the ingredients particularly mentioned above the formulations may include other agents conventional in the art having regard to the type of formulation in question. Of the possible formulations, sterile pyrogen-free aqueous and non-aqueous solutions are preferred.

The doses may be administered sequentially, eg. at daily, weekly or monthly intervals, or in response to a specific need of the patient. Preferred routes of administration are oral delivery and injection, typically parenteral or intramuscular injection or intratumoural injection.

The exact dosage regime will, of course, need to be determined by individual clinicians for individual patients and this, in turn, will be controlled by the exact nature of compound of formula (I) but some general guidance can be given. Typical dosage ranges generally will be those described above which may be administered in single or multiple doses. Other doses may be used according to the condition of the patient and other factors at the discretion of the physician.

The following Examples illustrate the invention.

Example 1: Preparation of 6-amino-3-(chloromethyl)-1-[(5',6',7'-trimethoxyvindol-2'-yl)carbonyl]indoline.

(a) t-Butyl 4-chloro-3-nitrobenzoate.

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A solution of 4-chloro-3-nitrobenzoic acid (10.03 g, 50 mmol), SOCl_2 (4.4 mL, 60 mmol) and DMF (4 drops) in 1,2-dichloroethane (150 mL) was stirred under reflux for 14 h, cooled and evaporated. The resulting crude acid chloride was dissolved in THF (100 mL), cooled to 0°C, and a solution of potassium t-butoxide (5.57 g, 50 mmol) in THF (150 mL) was added dropwise over 30 min under nitrogen. The mixture was stirred a further 15 min at 0°C, diluted with aqueous NaHCO_3 and extracted with EtOAc (x2), and the extracts were dried (Na_2SO_4) and evaporated. Flash chromatography of the residue on silica gel (petroleum ether/EtOAc; 30:1) gave t-butyl 4-chloro-3-nitrobenzoate as a white crystalline solid (11.45 g, 89%), mp (petroleum ether) 70-71 °C. ^1H NMR (CDCl_3) δ 8.42 (d, J = 2.0 Hz, 1 H, H-2), 8.11 (dd, J = 8.4, 2.0 Hz, 1 H, H-6), 7.61 (d, J = 8.4 Hz, 1 H, H-5), 1.61 (s, 9 H, t-Bu). Anal. Calculated for $\text{C}_{11}\text{H}_{12}\text{ClNO}_4$: 51.3; H, 4.7; N, 5.4; Cl, 13.8. Found: C, 51.6; H, 4.8; N, 5.4; Cl, 14.0%.

(b) Dimethyl (4-carboxy-2-nitrophenyl)malonate.

Sodium hydride (13.5 g of a 60% dispersion in oil, 0.34 mol) was washed with petroleum ether (x3) under nitrogen and suspended in dry THF (400 mL). A solution of dimethyl malonate (40.4 mL, 0.35 mol) in THF (50 mL) was added dropwise over 45 min with water-bath cooling, keeping the internal temperature below 30 °C, and the resulting gel was broken up with more dry THF (300 mL). The above t-butyl 4-chloro-3-nitrobenzoate (21.7 g, 84 mmol) was added and the mixture was stirred at reflux under nitrogen for 15 h. The red-brown solution was cooled, poured into water, and aqueous HCl (2 N, ca. 60 mL) added slowly until the red nitronate colour was dispersed. The THF was evaporated and the aqueous phase extracted with CH_2Cl_2 (x3), the extracts were dried (Na_2SO_4) and evaporated. Formic acid (100 mL) was added to the residue and the mixture was stirred at 50 °C for 4 h (when tlc analysis showed no remaining t-butyl ester). The formic acid was evaporated and the residue was taken up in EtOAc and washed with water (x3). The organic layer was extracted with aqueous NaHCO_3 (x2), and the aqueous phase was acidified (conc. HCl), and extracted with CH_2Cl_2 (x2). The organic layer was dried (Na_2SO_4), evaporated, and the resulting cream solid recrystallized from benzene (ca. 250 mL) to give dimethyl (4-carboxy-2-nitrophenyl)malonate as cream prisms (21.8 g, 87%), mp

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147-149 °C. ¹H NMR ((CD₃)₂SO) δ 13.77 (br s, 1 H, CO₂H), 8.52 (d, J = 1.7 Hz, 1 H, H-3), 8.28 (dd, J = 8.1, 1.7 Hz, 1 H, H-5), 7.70 (d, J = 8.1 Hz, 1 H, H-6), 5.62 (s, 1 H, ArCH), 3.71 (s, 6 H, CO₂Me); ¹³C NMR δ 166.9, 165.1 (COOMe, COOH), 148.2, 134.0, 133.1, 132.3, 132.1, 125.5 (C-1,2,3,4,5,6), 54.3 (ArCH), 52.9 (OMe). Anal. Calculated for C₁₂H₁₁NO₈: C, 48.5; H, 3.7; N, 4.7. Found: C, 48.7; H, 3.5; N, 4.7%.

(c) Dimethyl [4-(benzyloxycarbonyl)-amino-2-nitrophenyl]malonate.

A solution of the above malonate (3.44 g, 11.6 mmol), SOCl₂ (1.0 mL, 13.9 mmol) and DMF (4 drops) in 1,2-dichloroethane (60 mL) was stirred under reflux for 1 h, cooled and evaporated. The residue was dissolved in Me₂CO (30 mL) and added dropwise over 10 min to a vigorously stirred solution of sodium azide (2.26 g, 35 mmol) in water (30 mL) and acetone (100 mL) at 0 °C. After a further 30 min at 0 °C, EtOAc (100 mL) was added, most of the Me₂CO was evaporated, and the EtOAc layer was washed with water, dried (Na₂SO₄), and evaporated. The residue was dissolved in dry toluene (35 mL) and stirred at reflux for 40 min. Benzyl alcohol (2.2 mL, 21 mmol) was added to the cooled solution and the mixture stirred at 20 °C for 2 h [until a sample spotted on a tlc plate no longer showed the formation of yellow dimethyl (4-amino-2-nitrophenyl)malonate]. The mixture was then evaporated and the residue was distilled in a Kugelrohr (1 mm Hg, 90 °C) to remove excess benzyl alcohol. Flash chromatography on silica gel, eluting with petroleum ether/EtOAc (3:1) gave dimethyl [4-(benzyloxycarbonyl)-amino-2-nitrophenyl]malonate as a yellow oil (3.77 g, 81%). ¹H NMR (CDCl₃) δ 8.16 (d, J = 2.3 Hz, 1 H, H-3), 7.59 (dd, J = 8.5, 2.3 Hz, 1 H, H-5), 7.42-7.33 (m, 6 H, H-6 and Ph), 7.11 (s, 1 H, NH), 5.25 (s, 1 H, ArCH), 5.22 (s, 2 H, OCH₂Ph), 3.78 (s, 6 H, CO₂Me); ¹³C NMR δ 167.9, (CO₂Me), 152.8 (NCO₂), 149.0, 139.1, 135.4, 131.9, 128.7, 128.6, 128.4, 122.7, 121.9, 114.6 (aromatic C), 67.6 (OCH₂Ph), 53.5 (ArCH), 53.2 (OMe); MS (DEI) m/z 402 (2%, M⁺), 91 (100%, C₇H₇); HRMS calcd. for C₁₉H₁₈N₂O₈ 402.10631, found 402.10594.

(d) 2-[4-(Benzyloxycarbonyl)amino-2-nitrophenyl]propane-1,3-diol.

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A solution of the above 2-nitrophenylmalonate (3.12 g, 7.75 mmol) in THF (80 mL) was added dropwise over 30 min to a solution of diisobutylaluminium hydride (93 mL of a 1M solution in hexanes, 93 mmol) in THF (100 mL) under nitrogen, with cooling in an ice-salt bath (maintaining the internal temperature at -7 to 0 °C). The mixture was allowed to warm to 20 °C over 1 h, then poured into ice-cold aqueous HCl (3 N, 260 mL). The THF was evaporated, the aqueous residue was extracted with EtOAc (x3), and the extracts dried (Na₂SO₄) and evaporated. Dry column chromatography on silica gel, eluting with EtOAc/petroleum ether (1:3 then 1:1 then 2:1) gave recovered dimethyl [4-(benzyloxycarbonyl)amino-2-nitrophenyl]-malonate (0.42 g, 13%) and 2-[4-(benzyloxycarbonyl)amino-2-nitrophenyl]propane-1,3-diol as a light brown foam (1.35 g, 50 %). A sample of the latter was crystallized from CHCl₃, giving pale yellow flakes, mp 119-121 °C. ¹H NMR ((CD₃)₂SO) δ 10.15 (s, 1 H, NH), 7.97 (d, J = 2.2 Hz, 1 H, H-3), 7.60 (dd, J = 8.6, 2.2 Hz, 1 H, H-5), 7.49 (d, J = 8.6 Hz, 1 H, H-6), 7.45-7.33 (m, 5 H, Ph), 5.18 (s, 2 H, OCH₂Ph), 4.67 (t, J = 5.3 Hz, 2 H, OH), 3.73-3.66 (m, 2 H, CHHOH), 3.63-3.56 (m, 2 H, CHHOH), 3.23 (p, J = 6.4 Hz, 1 H, ArCH); ¹³C NMR δ 153.3 (NCO₂), 150.9, 137.8, 136.2, 129.0 (C-1,2,4 and i C of Ph), 129.9, 121.7, 112.3 (C-3,5,6), 128.4, 128.11, 128.09, (o, m, p C of Ph), 66.1 (OCH₂Ph), 61.8 (CH₂OH), 44.1 (ArCH). Anal. Calculated for C₁₇H₁₈N₂O₆: C, 59.0; H, 5.2; N, 8.1. Found: C, 58.9; H, 5.4; N, 8.3%.

(e)2-[2-Amino-4-(benzyloxycarbonyl)aminophenyl]propane-1,3-diol.

A solution of the above nitrodiol (1.02 g, 2.9 mmol) in EtOH (80 mL) with PtO₂ (0.12 g) was hydrogenated at 50 psi and 20 °C for 50 min, filtered through Celite, and evaporated. Dry column chromatography on silica gel, eluting with EtOAc/MeOH (20:1 then 10:1) gave the title compound as a very pale yellow oil (0.88 g, 94%). ¹H NMR ((CD₃)₂SO) δ 9.36 (s, 1 H, NH), 7.43-7.30 (m, 5 H, Ph), 6.82 (d, J = 2 Hz, 1 H, H-3), 6.81 (d, J = 8.3 Hz, 1 H, H-6), 6.58 (dd, J = 8.3, 2.1 Hz, 1 H, H-5), 5.12 (s, 2 H, OCH₂Ph), 4.82 (s, 2 H, NH₂ or OH), 4.50 (s, 2 H, NH₂ or OH), 3.69-3.62 (m, 2 H, CHHOH), 3.54-3.46 (m, 2 H, CHHOH), 2.83 (p, J = 6.2 Hz, 1 H, ArCH); ¹³C NMR δ 153.2 (NCO₂), 146.8, 137.2, 136.8, 120.4 (C-1,2,4 and i C of Ph), 128.3, 127.92, 127.86 (o, m, p C of Ph), 127.0,

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107.2, 105.2 (C-3,5,6), 65.3 (OCH₂Ph), 62.3 (CH₂OH), 43.2 (ArCH); MS (DEI) *m/z* 316 (30%, M⁺), 285 (30%, M - CH₂OH), 91 (100%, C₇H₇); HRMS calcd. for C₁₇H₂₀N₂O₄ 316.14231, found 316.14182.

(f) 2-[4-(Benzyloxycarbonyl)amino-2-(*t*-butyloxycarbonyl)-aminophenyl]propane-1,3-diol.

A solution of the above aminodiol (0.70 g, 2.21 mmol), di-*t*-butyldicarbonate (0.53 g, 2.4 mmol) and Na₂CO₃ (0.26 g, 2.4 mmol) in THF (120 mL) and water (60 mL) was stirred at 20 °C. More di-*t*-butyldicarbonate (2 x 0.53 g) was added after 5 and 8 days, with sufficient THF and water to maintain a single phase. After 14 days the THF was evaporated, the aqueous layer extracted with EtOAc (x2), and the organic extracts dried (Na₂SO₄) and evaporated. Dry column chromatography on silica gel, eluting with EtOAc/petroleum ether (2:1 then 4:1) gave the title compound as a white foam (0.78 g, 85%). ¹H NMR [(CD₃)₂SO] δ 9.67 (s, 1 H, NH), 8.62 (s, 1 H, NH), 7.60 (s, 1 H, H-3), 7.44-7.31 (m, 5 H, Ph), 7.19 (dd, *J* = 8.5, 1.7 Hz, 1 H, H-5), 7.08 (d, *J* = 8.5 Hz, 1 H, H-6), 5.14 (s, 2 H, OCH₂Ph), 4.84 (t, *J* = 4.7 Hz, 2 H, OH), 3.78-3.70 (m, 2 H, CHHOH), 3.54-3.45 (m, 2 H, CHHOH), 2.98 (p, *J* = 6.3 Hz, 1 H, ArCH), 1.45 (s, 9 H, *t*-Bu). ¹³C NMR δ 153.3 (resolves into two peaks on D₂O exchange, 2 x NCO₂), 137.1, 137.0, 136.7, 129.4 (C-1,2,4 and i C of Ph), 128.4, 127.91, 127.87 (o, m, p C of Ph), 127.4, 114.6, 114.4 (C-3,5,6), 78.8 (OCMe₃), 65.5 (OCH₂Ph), 62.8 (CH₂OH), 43.9 (ArCH), 28.1 (C(CH₃)₃); MS (DEI) *m/z* 416 (2%, M⁺), 91 (100%, C₇H₇); HRMS calcd. for C₂₂H₂₈N₂O₆ 416.19474, found 416.19544.

(g) 6-[(Benzyloxycarbonyl)amino]-1-(*t*-butyloxycarbonyl)-3-(hydroxymethyl)indoline.

Diethylazodicarboxylate (0.47 mL, 3.0 mmol) was added dropwise over 5 min to a solution of the above *t*-butyloxycarbonyl diol (0.74 g, 1.78 mmol) and triphenylphosphine (0.84 g, 3.2 mmol) in THF (60 mL) under nitrogen and the mixture stirred at 20 °C. After 10 min the mixture was diluted with EtOAc, washed with aqueous NaCl, and the organic phase dried (Na₂SO₄) and evaporated. Dry column chromatography on silica gel, eluting with EtOAc/petroleum ether (1:2) gave an overlapping band of reduced diethylazodicarboxylate and title product. A small fraction of the

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product was obtained in a pure state as a very pale yellow oil.
 ^1H NMR ($(\text{CD}_3)_2\text{SO}$) δ 9.68 (s, 1 H, NH), 7.97 (br s, 1 H, H-7),
7.44-7.31 (m, 5 H, Ph), 7.11 (d, J = 8.1 Hz, 1 H, H-4), 6.98 (br
d, J = 8 Hz, 1 H, H-5), 5.13 (s, 2 H, OCH_2Ph), 4.90 (t, J = 5.0
5 Hz, 1 H, OH), 3.94 (apparent t, J = 10.3 Hz, 1 H, NCHH), 3.75 (dd,
 J = 11.3, 5.1 Hz, 1 H, NCHH), 3.61-3.54 (m, collapses to dd, J =
10.2, 4.7 Hz in D_2O exchange, 1 H, CHHOH), 3.41-3.28 (m, 2 H,
ArCHCHHOH), 1.51 (s, 9 H, t-Bu); ^{13}C NMR δ 153.2, 151.6 (2 x
10 NCO_2), 143 (br), 138.6, 136.7, 126.4 (C-6,8,9 and i C of Ph),
128.3, 127.96, 127.90 (o, m, p C of Ph), 124.4, 112.1, 105.0
(C-4,5,7), 79.7 (OCMe_3), 65.5, 63.9 (OCH_2Ph , CH_2OH), 51.4 (C-2),
41.2 (C-3), 28.0 ($\text{C}(\text{CH}_3)_3$); MS (DEI) m/z 398 (4%, M^+); HRMS calcd.
for $\text{C}_{22}\text{H}_{26}\text{N}_2\text{O}_5$ 398.18417, found 398.18402.

(h) 6-[(Benzyloxycarbonyl)amino]-1-(t-butyloxycarbonyl)-3-
15 [(methanesulfonyloxy)methyl]indoline.

Methanesulfonyl chloride (0.25 mL, 3.2 mmol) was added to a
solution of the mixture obtained from the previous reaction (ca.
1.8 mmol of alcohol) and Et_3N (0.50 mL, 3.6 mmol) in CH_2Cl_2 (60 mL)
at 0 °C, and the mixture was stirred for 15 min. Aqueous NaHCO_3
20 was added, the mixture was extracted with CH_2Cl_2 (x2) and the
extracts dried (Na_2SO_4) and evaporated. Flash chromatography on
silica gel, eluting with CHCl_3 .EtOAc (20:1 then 10:1) gave title
compound as a white foam (0.79 g, 93 % for two steps). ^1H NMR
(CDCl_3) δ 7.73 (s, 1 H, H-7), 7.41-7.31 (m, 5 H, Ph), 7.12 (d, J
25 = 8.1 Hz, 1 H, H-4), 6.73 (s, 1 H, H-5), 5.19 (s, 2 H, OCH_2Ph),
4.32 (dd, J = 9.9, 5.5 Hz, 1 H, CHHOSO_2Me), 4.18 (dd, J = 9.9, 8.1
Hz, 1 H, CHHOSO_2Me), 4.11-4.02 (m, 1 H, NCHH), 3.92-3.84 (m, 1 H,
NCHH), 3.72-3.62 (m, 1 H, H-3), 2.96 (s, 3 H, OSO_2Me), 1.56 (s, 9
H, t-Bu); ^{13}C NMR δ 153.2, 152.1 (2 x NCO_2), 143.9, 138.7, 136.0,
30 123.6 (C-6,8,9 and i C of Ph), 128.6, 128.3 (br) (o, m, p C of
Ph), 124.9, 112.7, 105.9 (C-4,5,7), 81.2 (OCMe_3), 71.0 (CH_2OSO_2),
67.0 (OCH_2Ph), 51.1 (C-2), 39.1 (C-3), 37.5 (OSO_2CH_3), 28.4
($\text{C}(\text{CH}_3)_3$); MS (DEI) m/z 476 (5%, M^+), 91 (100%, C_7H_7); HRMS calcd.
for $\text{C}_{23}\text{H}_{28}\text{N}_2\text{O}_7\text{S}$ 476.16172, found 476.16070.

35 (i) 6-[(Benzyloxycarbonyl)amino]-3-[(methanesulfonyloxy)methyl]-1-
[(5',6',7'-trimethoxyindol-2'-yl)carbonyl]indoline.

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The above benzyloxycarbonylaminoindoline (306 mg, 0.64 mmol) was stirred in HCl-saturated EtOAc (10 mL) at 20 °C for 1 h (until tlc indicated complete reaction) and the mixture was evaporated. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI, 0.37 g, 1.9 mmol) and 5,6,7-trimethoxyindole-2-carboxylic acid [Y. Fukuda et al, Tetrahedron, 1994, 50, 2793-2808] (161 mg, 0.64 mmol) in DMF (15 mL) were added to the crude indoline hydrochloride, and the mixture stirred at 20 °C under nitrogen for 22 h. The DMF was evaporated, the residue was dissolved in EtOAc and water, extracted once more with EtOAc, and the organic extracts were dried (Na₂SO₄) and evaporated. Dry column chromatography of the residue on silica gel, eluting with EtOAc/petroleum ether (1:1), gave title compound as a pale pink crystalline solid (209 mg, 53 %), mp (EtOAc/petroleum ether) 153-154 °C. ¹H NMR δ 11.44 (s, 1 H, indole NH), 9.85 (s, 1 H, carbamate NH), 8.38 (s, 1 H, H-7), 7.46-7.33 (m, 5 H, Ph), 7.33 (d, J = 8.2 Hz, 1 H, H-4), 7.25 (dd, J = 8.2, 1.8 Hz, 1 H, H-5), 7.03 (d, J = 1.9 Hz, 1 H, H-3'), 6.95 (s, 1 H, H-4'), 5.15 (s, 2 H, OCH₂Ph), 4.62 (apparent t, J = 10 Hz, 1 H, CH₂), 4.45 (dd, J = 9.8, 5.1 Hz, 1 H, CH₂), 4.35 (dd, J = 9.8, 7.2 Hz, 1 H, CH₂), 4.27 (dd, J = 10.9, 5.3 Hz, 1 H, CH₂), 3.93 (s, 3 H, OCH₃), 3.87-3.80 (m, 1 H, H-3), 3.81 (s, 3 H, OCH₃), 3.80 (s, 3 H, OCH₃), 3.18 (s, 3 H, OSO₂Me). ¹³C NMR δ 160.1, 153.3, 149.1, 144.0, 139.8, 139.1, 139.0, 136.6, 130.8, 124.6, 123.1 (C-6,8,9,2',5',6',7',8',9', i C of Ph, NCO, NCO₂, one peak not observed), 128.4, 128.0, 127.9 (o, m, p C of Ph), 125.3, 113.9, 107.7, 106.1, 98.0 (C-4,5,7,3',4'), 71.3 (CH₂OSO₂), 65.6 (OCH₂Ph), 61.0, 60.9, 55.9 (3 x OCH₃), 53.0 (C-2), 39.3 (C-3), 36.5 (OSO₂CH₃). Anal. Calculated for C₃₀H₃₁N₃O₉S.0.5EtOAc: C, 58.8; H, 5.4; N, 6.4. Found: C, 58.7; H, 5.3; N, 6.6%.

(j) 6-Amino-3-(chloromethyl)-1-[(5',6',7'-trimethoxyindol-2'-yl)carbonyl]indoline.

A solution of ammonium formate (0.24 g, 3.8 mmol) in water (12 mL) was added to the above indoline (233 mg, 0.38 mmol) and Pd/C (5%, 100 mg) in THF (50 mL) and the mixture was stirred at 20 °C. More Pd/C (30 mg) was added after 70 min, and after 100 min (tlc indicates complete reaction) the catalyst was filtered off and washed with EtOAc. The filtrate was diluted with aq. NaCl,

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extracted with EtOAc (x2), and the extracts dried (Na_2SO_4) and evaporated. Dry column chromatography (eluting with 2:1 EtOAc:petroleum ether) gave 6-amino-3-[(methanesulfonyloxy)-methyl]-1-[(5',6',7'-trimethoxyindol-2'-yl)carbonyl]indoline as a pale yellow foam (154 mg, 85 %). This mesylate (150 mg, 0.32 mmol) and LiCl (0.13 g, 3.2 mmol) were stirred in DMF (5 mL) at 70 °C under nitrogen for 80 min, and the DMF evaporated. The residue was dissolved in EtOAc and water, extracted once more with EtOAc, and the organic extracts dried (Na_2SO_4) and evaporated. Dry column chromatography, eluting with EtOAc/petroleum ether (1:1) gave the title compound of the invention (87 mg, 66 %), mp (EtOAc/ Et_2O) 173-174 °C. ^1H NMR [$(\text{CD}_3)_2\text{SO}$] δ 11.36 (d, J = 1.6 Hz, 1 H, NH), 7.44 (br s, 1 H, H-7), 7.05 (d, J = 8.0 Hz, 1 H, H-4), 6.96 (d, J = 2.1 Hz, 1 H, H-3'), 6.95 (s, 1 H, H-4'), 6.30 (dd, J = 8.0, 2.2 Hz, 1 H, H-5), 5.18 (s, 2 H, NH_2), 4.54 (dd, J = 10.8, 8.7 Hz, 1 H, NCHH), 4.20 (dd, J = 10.8, 4.4 Hz, 1 H, NCHH), 3.93 (s, 3 H, OCH_3), 3.91 (dd, J = 9.9, 3.5 Hz, 1 H, CHHCl), 3.81 (s, 3 H, OCH_3), 3.79 (s, 3 H, OCH_3), 3.74-3.60 (m, 2 H, CHCHHCl). ^{13}C NMR δ 159.9, 149.0, 144.2, 139.6, 139.0, 131.2, 125.1, 123.1, 118.8 (C-6,8,9,2',5',6',7',8',9', NCO, one peak not observed), 124.7, 109.5, 105.6, 102.9, 98.0 (C-4,5,7,3',4'), 61.0, 60.9, 55.9 (3 x OCH_3), 54.5 (C-2), 47.9 (CH_2Cl), 41.8 (C-3). Anal. Calculated for $\text{C}_{21}\text{H}_{22}\text{ClN}_3\text{O}_4$: C, 60.7; H, 5.3; N, 10.1. Found: C, 60.7; H, 5.4; N, 9.8%.

Example 2: Alternative synthesis of 6-amino-3-(chloromethyl)-1-[(5',6',7'-trimethoxyindol-2'-yl)carbonyl]indoline.

This example illustrates the route of synthesis outlined in Scheme 2.

(a) t-Butyl 2-chloro-5-nitrobenzoate.

A solution of 2-chloro-5-nitrobenzoic acid (10.14 g, 50.3 mmol), thionyl chloride (4.4 mL, 60 mmol) and DMF (4 drops) in 1,2-dichloroethane (150 mL) was stirred at reflux for 14 h, cooled and evaporated. The acid chloride was dissolved in THF (100 mL), cooled to 0 °C, and a solution of potassium t-butoxide (5.57 g, 50 mmol) in THF (150 mL) added dropwise over 30 min under nitrogen. The mixture was stirred a further 15 min at 0 °C, diluted with aq.

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NaHCO₃, extracted with EtOAc (x2), and the extracts dried (Na₂SO₄) and evaporated. Crystallisation from petroleum ether gave *t*-butyl 2-chloro-5-nitrobenzoate as a white crystalline solid (10.1 g, 78 %), mp 91.5-92 °C. ¹H NMR (CDCl₃) δ 8.59 (d, *J* = 2.8 Hz, 1 H, H-6), 8.24 (dd, *J* = 8.8, 2.8 Hz, 1 H, H-4), 7.63 (d, *J* = 8.8 Hz, 1 H, H-3), 1.65 (s, 9 H, *t*-Bu); ¹³C NMR (CDCl₃) δ 163.0 (CO₂*t*Bu), 146.1, 140.0, 133.3, (C-1,2,5), 132.0, 126.1, 126.0 (C-3,4,6), 84.0 (CMe₃), 28.1 (CH₃). Anal. Calculated for C₁₁H₁₂ClNO₄: C, 51.3; H, 4.7; N, 5.4; Cl, 13.8. Found: C, 51.3; H, 4.5; N, 5.6; Cl, 14.0%.

(b) Dimethyl (2-carboxy-4-nitrophenyl)malonate.

Sodium hydride (12.42 g of a 60% dispersion in oil, 310 mmol) was washed with petroleum ether (x3) under nitrogen and suspended in dry DMSO (250 mL). A solution of dimethyl malonate (37.3 mL, 330 mmol) in DMSO (50 mL) was added dropwise over 35 min with water-bath cooling. *t*-Butyl 2-chloro-5-nitrobenzoate (20.0 g, 78 mmol) was added and the mixture stirred at 70-80 °C under nitrogen for 4 h. The red-brown solution was cooled, poured into water (300 mL), and aq. HCl (2 N, 60 mL) added slowly until the red nitronate colour was dispersed. The mixture was extracted with CH₂Cl₂ (x3), the extracts were dried (Na₂SO₄) and evaporated. Formic acid (60 mL) was added to the residue and the mixture stirred at 20 °C for 15 h then 50 °C for 7 h (when tlc analysis showed no remaining *t*-butyl ester). The formic acid was evaporated and the residue taken up in EtOAc and washed with aq. NaCl (x3). The organic layer was extracted with aq. NaHCO₃ (x3), the aqueous phase acidified (c.HCl), and extracted with CH₂Cl₂ (x3). The organic phase was dried (Na₂SO₄), evaporated, and the residue recrystallised from benzene (two crops) to give dimethyl (2-carboxy-4-nitrophenyl)malonate as cream needles (20.27 g, 88%), mp 163-164 °C. ¹H NMR (CDCl₃) δ 8.99 (d, *J* = 2.5 Hz, 1 H, H-3), 8.44 (dd, *J* = 8.6, 2.5 Hz, 1 H, H-5), 8.4 (v br s, 1 H, CO₂H), 7.73 (d, *J* = 8.6 Hz, 1 H, H-6), 5.94 (s, 1 H, ArCH), 3.83 (s, 6 H, CO₂Me); ¹³C NMR δ 169.6, 167.8 (CO₂Me, CO₂H), 147.4, 141.4, 132.2, 129.7, 127.6, 126.8 (C-1,2,3,4,5,6), 54.4 (ArCH), 53.3 (OMe). Anal. Calculated for CH₁₁NO₈: C, 48.5; H, 3.7; N, 4.7. Found: C, 48.7; H, 3.7; N, 5.0%.

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(c) 3,3-Di(methoxycarbonyl)-6-nitro-2-indolone.

Triethylamine (8.64 mL, 62 mmol) was added to a solution of dimethyl (2-carboxy-4-nitrophenyl)malonate (18.43 g, 62 mmol) and diphenylphosphoryl azide (DPPA, 13.4 mL, 62 mmol) and the mixture stirred at reflux for 15 h. The orange-brown solution was cooled, concentrated to a small volume, and the solid filtered off, washing with 2N HCl then water. The solid was triturated with hot MeOH (ca. 80 mL) to give title compound as a pale yellow powder (15.4 g, 84%), mp 234-239 °C (dec.). ¹H NMR ((CD₃)₂SO) δ 11.43 (br s, 1 H, NH), 7.97 (dd, J = 8.3, 2.1 Hz, 1 H, H-5), 7.66 (d, J = 8.3 Hz, 1 H, H-4), 7.62 (d, J = 2.1 Hz, 1 H, H-7), 3.76 (s, 6 H, CO₂Me); ¹³C NMR δ 167.4, 163.9 (CO₂Me, CONH), 148.4, 143.7, 130.4 (C-6,8,9), 126.7, 117.7, 104.6 (C-4,5,7), 65.7 (C-3), 54.0 (OMe); IR (KBr) 1767 (CONH), 1736 (CO₂Me), 1524 (NO₂), 1348 (NO₂), 1246 cm⁻¹; MS (DEI) 294 (85%, M⁺), 250 (100%, M - CO₂); HRMS calcd. for C₁₂H₁₀N₂O₇, 294.04880, found 294.04851. Anal. Calculated for C₁₂H₁₀N₂O₇: C, 49.0; H, 3.4; N, 9.5. Found: C, 49.1; H, 3.4; N, 9.6%.

(d) 3,3-Di(methoxycarbonyl)-6-nitroindoline.

Borane-dimethylsulfide (6.35 mL, 63 mmol) was added to a suspension of 3,3-di(methoxycarbonyl)-6-nitro-2-indolone (10.38 g, 35.2 mmol) in THF (350 mL) under nitrogen, and the mixture stirred at reflux for 1 h. The pale yellow solution was cooled, MeOH (10 mL), then H₂O (10 mL), then aq. HCl (2 N, 50 mL) added, and the mixture stirred at 20 °C for a few minutes. The THF was evaporated and the aqueous residue extracted with EtOAc (x2). The extracts were dried (Na₂SO₄) and evaporated, and the resulting orange solid triturated with CH₂Cl₂ (2 x 100 mL) at 20 °C and filtered to remove most of the 3-(methoxycarbonyl)-6-nitroindole impurity. The CH₂Cl₂ solution was filtered through a short column of silica, eluting with more CH₂Cl₂, the solvent evaporated, and the resulting yellow solid recrystallised from MeOH to give title compound as a yellow crystalline solid (5.11 g, 52%), mp 139.5-140.5 °C. ¹H NMR (CDCl₃) δ 7.64 (dd, J = 8.3, 2.1 Hz, 1 H, H-5), 7.56 (d, J = 8.3 Hz, 1 H, H-4), 7.41 (d, J = 2.1 Hz, 1 H, H-7), 4.24 (d, J = 1.9 Hz, 2 H, NHCH₂), 4.16 (br s, 1 H, NH), 3.82 (s, 6 H, CO₂Me); ¹³C NMR δ 168.7 (CO₂Me), 151.7, 149.8, 130.8 (C-6,8,9), 127.2, 114.2, 104.2 (C-4,5,7), 62.7 (C-3), 54.1 (C-2), 53.6 (OMe); IR (KBr) 3351 (NH), 1732 (CO₂Me), 1535 (NO₂),

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1351 (NO_2), 1279 cm^{-1} . Anal. Calculated for $\text{C}_{12}\text{H}_{12}\text{N}_2\text{O}_6$: C, 51.4; H, 4.3; N, 10.0. Found: C, 51.4; H, 4.4; N, 10.0%.

(e) 1-(t-Butyloxycarbonyl)-3,3-di(methoxycarbonyl)-6-nitroindoline.

5 A solution of 3,3-di(methoxycarbonyl)-6-nitroindoline (3.04 g, 10.8 mmol), di-t-butyldicarbonate (3.55 g, 16.3 mmol) and 4-dimethylaminopyridine (70 mg, 0.5 mmol) in THF (100 mL) was stirred at 20 °C for 2 h then at reflux for 10 min (until tlc analysis showed complete conversion). The THF was evaporated and
10 the residue purified by dry column chromatography, eluting with EtOAc/petroleum ether (1:4), to give title compound as a pale yellow foam (4.10 g, 99%). A sample was crystallised from MeOH, giving very pale yellow needles, mp 131.5-132.5 °C. ^1H NMR (CDCl_3) δ 8.70, 8.34 (2 x br s, 1 H, H-7), 7.89 (dd, J = 8.5, 2.2 Hz, 1 H, H-5), 7.66 (d, J = 8.5 Hz, 1 H, H-4), 4.59 (s, 2 H, NCH_2), 3.83
15 (s, 6 H, CO_2Me), 1.60 (s, 9 H, t-Bu); ^{13}C NMR δ 168.1 (CO_2Me), 151.2, 149.7 (C-6,8,9, NCO_2 , two peaks not observed), 127.4, 117.6, 110.0 (C-4,5,7), 82.5 (OCMe_3), 61 (br, C-3), 54.6 (C-2), 53.9 (OMe), 28.3 ($\text{C}(\text{CH}_3)_3$). Anal. Calculated for $\text{C}_{17}\text{H}_{20}\text{N}_2\text{O}_8$: C, 53.7; H, 5.3; N, 7.4. Found: C, 53.6; H, 5.4; N, 7.5%.

(f) 1-(t-Butyloxycarbonyl)-3-(methoxycarbonyl)-6-nitroindoline.

NaOMe (4.8 mL of a 1.28 M solution in MeOH, 6.1 mmol) was added dropwise to a solution of 1-(t-butyloxycarbonyl)-3,3-di(methoxycarbonyl)-6-nitroindoline (2.11 g, 5.55 mmol) in THF (100 mL)
25 under nitrogen at 20 °C, immediately giving an intense purple colour. After 5 min trifluoroacetic acid (0.51 mL, 6.7 mmol) was added in one portion, causing the nitronate colour to disperse. The pale yellow solution was diluted with aq. NaCl, extracted with EtOAc, and the extracts dried (Na_2SO_4) and evaporated to give
30 crude title compound as a pale yellow oil. This compound showed signs of air oxidation on standing at room temperature, so was not further purified but used directly in the next step. ^1H NMR (CDCl_3) δ 8.67, 8.34 (2 x br s, 1 H, H-7), 7.85 (dd, J = 8.2, 2.1 Hz, 1 H, H-5), 7.49 (d, J = 8.2 Hz, 1 H, H-4), 4.48 (dd, J = 10.5, 5.4 Hz, 1 H, NCH_2CH), 4.28 (dd, J = 10.5, 5.3 Hz, 1 H, NCH_2CH),
35 4.22 (t, J = 10.6 Hz, 1 H, NCH_2CH), 3.82 (s, 3 H, CO_2Me), 1.60 (s, 9 H, t-Bu); ^{13}C NMR δ 170.5 (CO_2Me), 151.6, 149.1 (C-6,8,9, NCO_2 ,

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two peaks not observed), 125.5, 117.7, 109.9 (C-4,5,7), (OCMe₃ peak not observed), 53.1 (OMe), 50.3 (C-2), 44.4 (C-3), 28.3 (C(CH₃)₃). HRMS calculated for C₁₅H₁₈N₂O₆: 322.11649. Found: 322.11627.

5 (g) 1-(t-Butyloxycarbonyl)-3-(hydroxymethyl)-6-nitroindoline.

The crude monoester from the above procedure was dissolved in THF (80 mL) and added dropwise over 30 min to a solution of diisobutylaluminium hydride (22.2 mL of a 1 M solution in toluene, 22.2 mmol) in THF (100 mL) under nitrogen at 0 °C. The yellow-orange solution was stirred at this temperature for 25 min, then poured into ice-cold aqueous HCl (2 N, 100 mL) and extracted with EtOAc (x2). The extracts were dried (Na₂SO₄), evaporated, and the residue purified by dry column chromatography, eluting with EtOAc/petroleum ether (1:1) to give as a yellow-orange solid (1.30 g, 80% from the diester). A sample was recrystallised from benzene, giving a yellow crystalline solid, mp 168.5-169 °C. ¹H NMR (CDCl₃) δ 8.64, 8.31 (2 x br s, 1 H, H-7), 7.83 (dd, J = 8.2, 2.3 Hz, 1 H, H-5), 7.34 (d, J = 8.2 Hz, 1 H, H-4), 4.16 (dd, J = 11.4, 10.3 Hz, 1 H, H-2), 3.96 (dd, J = 11.4, 5.4 Hz, 1 H, H-2), 3.84 (d, J = 6.2 Hz, 2 H, CH₂OH), 3.62-3.54 (m, 1 H, H-3), 1.91 (br s, 1 H, OH), 1.59 (s, 9 H, t-Bu); ¹³C NMR δ 152.0, 148.6, 144 (br), 139 (br) (C-6,8,9, NCO₂), 124.6, 117.7, 109.7 (C-4,5,7), 82 (br, OCMe₃), 64.8 (CH₂OH), 51.3 (C-2), 41.8 (C-3), 28.3 (C(CH₃)₃). Anal. Calculated for C₁₄H₁₈N₂O₅: C, 57.1; H, 6.2; N, 9.5. Found: C, 57.2; H, 6.2; N, 9.5%.

25 (h) 1-(t-Butyloxycarbonyl)-3-(chloromethyl)-6-nitroindoline.

Methanesulfonyl chloride (0.57 mL, 7.4 mmol) was added dropwise to a solution of 1-(t-butyloxycarbonyl)-3-(hydroxymethyl)-6-nitroindoline (1.21 g, 4.11 mmol) and Et₃N (1.15 mL, 8.2 mmol) in CH₂Cl₂ (70 mL) at 0 °C, and the pale yellow solution stirred for 5 min. Water was added, the mixture was extracted with CH₂Cl₂ (x2), and the extracts dried (Na₂SO₄) and evaporated. The crude mesylate was dissolved in DMF (10 mL) with LiCl (0.70 g, 16 mmol) and the mixture stirred at 80 °C under nitrogen for 1 h. The DMF was evaporated, the residue dissolved in EtOAc and water, extracted once more with EtOAc, and the organic extracts dried (Na₂SO₄) and evaporated. Dry column chromatography, eluting with EtOAc/petroleum ether (1:9), gave title compound as pale yellow

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foam (1.12 g, 87%). A sample was crystallised from benzene-petroleum ether to give a yellow crystalline solid, mp 111-111.5 °C. ¹H NMR (CDCl₃) δ 8.67, 8.34 (2 x br s, 1 H, H-7), 7.86 (dd, *J* = 8.2, 2.2 Hz, 1 H, H-5), 7.35 (d, *J* = 8.2 Hz, 1 H, H-4), 4.22 (dd, *J* = 11.6, 9.6 Hz, 1 H, H-2), 4.04-3.97 (m, 1 H, H-2), 3.82-3.74 (m, 2 H, CHHCl, H-3), 3.68-3.62 (m, 1 H, CHHCl), 1.60 (s, 9 H, t-Bu); ¹³C NMR δ 151.8, 149.0, 144 (br), 138 (br) (C-6,8,9, NCO₂), 124.6, 117.8, 110.0 (C-4,5,7), 82.0 (OCMe₃), 52.4 (C-2), 46.3 (CH₂Cl), 41.7 (C-3), 28.4 (C(CH₃)₃). Anal. Calculated for C₁₄H₁₈ClN₂O₄: C, 53.8; H, 5.5; N, 11.3; Cl, 11.3. Found: C, 54.0; H, 5.5; N, 9.1; Cl, 11.5%.

(i) 3-(Chloromethyl)-6-nitro-1-[(5',6',7'-trimethoxyindol-2'-yl)carbonyl]indoline.

1-(t-Butyloxycarbonyl)-3-(chloromethyl)-6-nitroindoline (134 mg, 0.43 mmol) was stirred in HCl-saturated EtOAc (8 mL) at 20 °C for 2.5 h (until tlc indicated complete reaction) and the mixture evaporated. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI, 0.25 g, 1.29 mmol) and 5,6,7-trimethoxyindole-2-carboxylic acid (108 mg, 0.43 mmol) in DMF (5 mL) were added, and the mixture stirred at 20 °C. Over 10 min the colour faded from orange to a light yellow-brown solution. After 2 h the DMF was evaporated, the residue dissolved in EtOAc and water, extracted once more with EtOAc, and the organic extracts were dried (Na₂SO₄) and evaporated. The yellow-orange solid was recrystallised from EtOAc, giving title compound as a yellow crystalline solid (96 mg, 50%), mp 187-188 °C. The mother liquors were purified by dry column chromatography, eluting with EtOAc/petroleum ether (1:2), to give a second crop of the product (46 mg, combined yield 74%). ¹H NMR (CDCl₃) δ 9.48 (s, 1 H, NH), 9.08 (d, *J* = 2.2 Hz, 1 H, H-7), 7.92 (dd, *J* = 8.2, 2.2 Hz, 1 H, H-5), 7.38 (d, *J* = 8.6 Hz, 1 H, H-4), 6.94 (d, *J* = 2.4 Hz, 1 H, H-3'), 6.81 (s, 1 H, H-4'), 4.73 (dd, *J* = 10.5, 9.6 Hz, 1 H, H-2), 4.52 (dd, *J* = 10.5, 5.2 Hz, 1 H, H-2), 4.07 (s, 3 H, OCH₃), 4.02-3.95 (m, 1 H, H-3), 3.95 (s, 3 H, OCH₃), 3.89 (s, 3 H, OCH₃), 3.87 (dd, *J* = 11.2, 4.7 Hz, 1 H, CHHCl), 3.71 (dd, *J* = 11.2, 8.1 Hz, 1 H, CHHCl); ¹³C NMR δ 160.4 (NCO), 150.3, 148.7, 144.7,

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140.7, 138.8, 137.7, 128.8, 125.8, 123.5 (C-6,8,9, 2',5',6',7',8',9'), 124.2, 119.5, 112.9, 106.9, 97.5 (C-4,5,7, 3',4'), 61.4, 61.1, 56.2 (3 x OCH₃), 54.3 (C-2), 46.1 (CH₂Cl), 43.2 (C-3). Anal. Calculated for C₂₁H₂₀ClN₃O₆: C, 56.6; H, 4.5; N, 9.4; Cl, 8.0. Found: C, 54.8; H, 4.5; N, 9.3; Cl, 8.1%.

(j) 6-Amino-3-(chloromethyl)-1-[(5',6',7'-trimethoxyindol-2'-yl)carbonyl]indoline.

A solution of 3-(chloromethyl)-6-nitro-1-[(5',6',7'-trimethoxyindol-2'-yl)carbonyl]indoline (57 mg, 0.13 mmol) in THF (8 mL) with PtO₂ (25 mg) was hydrogenated at 45 psi and 20 °C for 20 min, filtered through Celite, and evaporated. Dry column chromatography, eluting with EtOAc/petroleum ether (1:1), gave title compound of the invention (53 mg, 100 %), identical (tlc, ¹H NMR) to the material prepared in Example 1 above.

Example 3: 3-(Chloromethyl)-6-[(4''-nitrobenzyloxycarbonyl)amino]-1-[(5',6',7'-trimethoxyindol-2'-yl)carbonyl]indoline.

4-Nitrobenzyl chloroformate (27 mg, 1.7 mmol) was added to a solution of 1 (30 mg, 0.07 mmol) in THF (4 mL) at 0 °C, and the mixture was stirred at this temperature for 30 min. Water was added, the mixture was extracted with EtOAc (x2), and the extracts dried (Na₂SO₄) and evaporated. Flash chromatography, eluting with EtOAc/petroleum ether (1:1) gave title compound (33 mg, 77%) as a pale yellow powder, mp (trituration with Et₂O from CHCl₃) 199-200 °C). ¹H NMR [(CD₃)₂SO] δ 11.43 (s, 1 H, indole NH), 9.97 (s, 1 H, carbamate NH), 8.35 (br s, 1 H, H-7), 8.28 (br d, J = 8.8 Hz, 2 H, H-3'',5''), 7.70 (br d, J = 8.8 Hz, 2 H, H-2'',6''), 7.34 (d, J = 8.2 Hz, 1 H, H-4), 7.24 (dd, J = 8.2, 1.8 Hz, 1 H, H-5), 7.02 (d, J = 2.0 Hz, 1 H, H-3'), 6.96 (s, 1 H, H-4'), 5.30 (s, 2 H, OCH₂Ar), 4.63 (dd, J = 10.8, 8.7 Hz, 1 H, NCHH), 4.28 (dd, J = 10.8, 4.5 Hz, 1 H, NCHH), 4.02-3.95 (m, 1 H, CHHCl), 3.92 (s, 3 H, OCH₃), 3.86-3.76 (m, 2 H, CHCHHCl), 3.81 (s, 3 H, OCH₃), 3.79 (s, 3 H, OCH₃). Anal. Calculated for C₂₉H₂₇ClN₄O₈: C, 58.5; H, 4.6; N, 9.4. Found: C, 58.4; H, 4.3; N, 13.7%.

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Example 4: Preparation of 2-[[3-(chloromethyl)-6-nitroindolin-1-yl]carbonyl]-N-[2-(2-pyridinyl)ethyl]indole-6-carboxamide hydrochloride.

A solution of *t*-butyl 4-formylbenzoate (8.52 g, 41 mmol) and methyl azidoacetate [M. S. Allen, L. K. Hamaker, A. J. La Loggia, J. M. Cook, *Synth. Commun.*, 1992, 22, 2077-2102] (28.5 g, 248 mmol) in dry MeOH (200 mL) was added dropwise over 1h to a solution of NaOMe (60 mL of a 3.3M solution in MeOH, 197 mmol) under nitrogen with cooling in an ice-salt bath. The yellow suspension was stirred for a further 1h at 0 °C, allowed to stand at 4 °C for 15h, then diluted with water and the solid filtered off and dried in the dark. A solution of this crude azidocinnamate (8.76 g, 29 mmol) in dry xylene (400 mL) was added dropwise over 2 h to xylene (100 mL) at reflux under nitrogen, the solution stirred at reflux for a further 1 h, then cooled and evaporated. Recrystallisation from MeOH gave *t*-butyl 2-(methoxycarbonyl)indole-6-carboxylate as a white solid (4.72 g, 42%), mp 189-190.5 °C. ¹H NMR [(CD₃)₂SO] δ 12.26 (s, 1 H, NH), 8.08 (s, 1 H, H-3 or H-7), 7.73 (d, *J* = 8.4 Hz, 1 H, H-4), 7.61 (dd, *J* = 8.4, 1.3 Hz, 1 H, H-5), 7.21 (d, *J* = 1.3 Hz, 1 H, H-3 or H-7), 3.89 (s, 3 H, CO₂Me), 1.56 (s, 9 H, CO₂tBu); ¹³C NMR δ 165.5 (CO₂tBu), 161.4 (CO₂Me), 136.5, 130.0, 129.9, 127.3 (C-2,6,8,9), 121.9, 120.3, 114.4, 107.5 (C-3,4,5,7), 80.4 (OCMe₃), 52.1 (OCH₃), 27.8 (tBu). Anal. (C₁₅H₁₇NO₄) C,H,N.

t-Butyl 2-(methoxycarbonyl)indole-6-carboxylate (4.74 g, 17.2 mmol) was stirred in formic acid (35 mL) at 80 °C for 20 min, cooled, diluted with water, and the solid filtered off and dried to give 2-(methoxycarbonyl)indole-6-carboxylic acid (3.78 g, 100%) as a white solid, mp 270.5-271.5 °C. ¹H NMR [(CD₃)₂SO] δ 12.80 (br s, 1 H, CO₂H), 12.31 (s, 1 H, NH), 8.13 (s, 1 H, H-3 or H-7), 7.75 (d, *J* = 8.4 Hz, 1 H, H-4), 7.66 (dd, *J* = 8.4, 1.4 Hz, 1 H, H-5), 7.23 (dd, *J* = 2.0, 0.8 Hz, 1 H, H-3 or H-7), 3.91 (s, 3 H, CO₂Me); ¹³C NMR δ 167.8 (CO₂H), 161.4 (CO₂Me), 136.5, 129.8, 129.7, 126.6 (C-2,6,8,9), 121.9, 120.6, 114.7, 107.5 (C-3,4,5,7), 52.0 (OCH₃). Anal. (C₁₁H₉NO₄) C,H,N.

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A solution of 2-(methoxycarbonyl)indole-6-carboxylic acid (290 mg, 1.32 mmol) and 1,1'-carbonyldiimidazole (257 mg, 1.59 mmol) in THF (20 mL) was stirred at reflux for 1 h then cooled and evaporated. A solution of 2-(2-aminoethyl)pyridine (0.19 mL, 1.6 mmol) in DMF (5 mL) was added and the mixture stirred at 80 °C for 2 h, then cooled and evaporated. Trituration with hot EtOAc gave 2-(methoxycarbonyl)-N-[2-(2-pyridinyl)ethyl]indole-6-carboxamide as a white solid (360 mg, 84%), mp 191-193 °C. ¹H NMR [(CD₃)₂SO] δ 12.24 (s, 1 H, indole NH), 8.61 (t, J = 5.5 Hz, 1 H, amide NH), 8.54-8.50 (m, 1 H, H-6'), 7.97 (s, 1 H, H-3 or H-7), 7.71 (td, J = 7.6, 1.8 Hz, 1 H, H-4'), 7.70 (d, J = 8.6 Hz, 1 H, H-4), 7.55 (dd, J = 8.6, 1.4 Hz, 1 H, H-5), 7.29 (d, J = 7.8 Hz, 1 H, H-3'), 7.23 (ddd, J = 7.4, 4.8, 0.8 Hz, 1 H, H-5'), 7.20 (s, 1 H, H-3 or H-7), 3.90 (s, 3 H, CO₂Me), 3.64 (q, J = 6.8 Hz, 2 H, NHCH₂CH₂py), 3.03 (t, J = 7.4 Hz, 2 H, NHCH₂CH₂py); ¹³C NMR δ 166.6 (CONH), 161.5 (CO₂Me), 159.2 (C-2'), 136.7, 131.0, 129.1, 128.5 (C-2,6,8,9), 149.0, 136.4, 123.1, 121.6, 121.4, 118.9, 112.2, 107.5 (C-3,4,5,7,3',4',5',6'), 51.9 (OCH₃), 39.3, 37.3 (NHCH₂CH₂py). Anal. (C₁₈H₁₇N₃O₃) C, H, N.

A mixture of 2-(methoxycarbonyl)-N-[2-(2-pyridinyl)ethyl]indole-6-carboxamide (326 mg, 1.01 mmol) and KOH (68 mg, 1.21 mmol) in THF (20 mL) and H₂O (3 mL) was stirred at 20 °C for 15 h then at 65 °C for 3h, cooled and neutralised with 2N HCl (0.61 mL, 1.2 mmol). The THF was evaporated, the aqueous residue allowed to cool, and the solid filtered off and dried to give 2-carboxy-N-[2-(2-pyridinyl)ethyl]indole-6-carboxamide as a white solid (258 mg, 83%), mp 198-199 °C. ¹H NMR [(CD₃)₂SO] δ 13.14 (br s, 1 H, CO₂H), 12.07 (s, 1 H, indole NH), 8.59 (t, J = 5.6 Hz, 1 H, amide NH), 8.54-8.51 (m, 1 H, H-6'), 7.95 (s, 1 H, H-3 or H-7), 7.71 (td, J = 7.7, 1.7 Hz, 1 H, H-4'), 7.68 (d, J = 8.5 Hz, 1 H, H-4), 7.53 (dd, J = 8.5, 1.4 Hz, 1 H, H-5), 7.29 (d, J = 7.8 Hz, 1 H, H-3'), 7.23 (ddd, J = 7.4, 4.7, 0.8 Hz, 1 H, H-5'), 7.13 (d, J = 1.2 Hz, 1 H, H-3 or H-7), 3.64 (q, J = 6.8 Hz, 2 H, NHCH₂CH₂py), 3.02 (t, J = 7.4 Hz, 2 H, NHCH₂CH₂py); ¹³C NMR δ 166.7 (CONH), 162.5 (CO₂H), 159.2 (C-2'), 136.5, 130.6, 130.5, 128.7 (C-2,6,8,9), 149.0, 136.4, 123.1, 121.4, 118.7, 112.2, 107.0 (one peak doubled or not observed, C-3,4,5,7,3',4',5',6'), 39.3, 37.3 (NHCH₂CH₂py). Anal. (C₁₇H₁₅N₃O₃) C, H, N.

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1-(*t*-Butoxycarbonyl)-3-(chloromethyl)-6-nitroindoline (248 mg, 0.79 mmol) was stirred in HCl-saturated dioxane (8 mL) at 20 °C for 40 min (until tlc indicated complete reaction) and the mixture evaporated. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.30 g, 1.58 mmol) and 2-carboxy-*N*-[2-(2-pyridinyl)ethyl]indole-6-carboxamide (245 mg, 0.79 mmol) in DMF (20 mL) were added and the orange solution stirred at 20 °C. After 24 h the DMF was evaporated, and the residue triturated with EtOAc and aqueous NaHCO₃. The solid was filtered off and recrystallised from MeOH to give 2-[[3-(chloromethyl)-6-nitroindolin-1-yl]carbonyl]-*N*-[2-(2-pyridinyl)ethyl]indole-6-carboxamide as a yellow solid (267 mg, 67%), mp 204-207 °C. ¹H NMR [(CD₃)₂SO] δ 12.15 (s, 1 H, indole NH), 8.99 (d, *J* = 2.1 Hz, 1 H, H-7), 8.61 (t, *J* = 5.5 Hz, 1 H, amide NH), 8.55-8.52 (m, 1 H, H-6''), 8.04 (dd, *J* = 8.3, 2.3-Hz, 1 H, H-5), 8.02 (s, 1 H, H-3' or H-7'), 7.75 (d, *J* = 8.6 Hz, 1 H, H-4 or H-4'), 7.74 (d, *J* = 8.3 Hz, 1 H, H-4 or H-4'), 7.71 (dd, *J* = 7.6, 1.9 Hz, 1 H, H-4''), 7.56 (dd, *J* = 8.5, 1.3 Hz, 1 H, H-5'), 7.31 (d, *J* = 7.7 Hz, 1 H, H-3''), 7.28 (d, *J* = 1.4 Hz, 1 H, H-3' or H-7'), 7.23 (ddd, *J* = 7.2, 4.9, 0.8 Hz, 1 H, H-5''), 4.86 (t, *J* = 10.1 Hz, 1 H, H-2), 4.51 (dd, *J* = 10.7, 5.1 Hz, 1 H, H-2), 4.19-4.06 (m, 3 H, H-3 and CH₂Cl), 3.66 (q, *J* = 6.7 Hz, 2 H, NHCH₂CH₂py), 3.04 (t, *J* = 7.4 Hz, 2 H, NHCH₂CH₂py); ¹³C NMR δ 166.7 (CONH), 160.4 (CON), 159.2 (C-2''), 147.6, 144.6, 139.9, 135.7, 131.8, 130.8, 128.9 (C-6,8,9,2',6'',8'',9''), 149.0, 136.4, 123.1, 121.4 (C-3'',4'',5'',6''), 125.5, 121.6, 119.4, 118.8, 112.0, 111.2, 106.1 (C-4,5,7,3',4',5',7'), 54.0 (C-2), 47.0 (CH₂Cl), 42.0 (C-3), 39.3, 37.3 (NHCH₂CH₂py). A sample was converted to the hydrochloride salt and triturated with hot EtOH to give 2-[[3-(chloromethyl)-6-nitroindolin-1-yl]carbonyl]-*N*-[2-(2-pyridinyl)ethyl]indole-6-carboxamide hydrochloride as a pale yellow powder, mp 250-255 °C (dec.). Anal. (C₂₆H₂₂ClN₅O₄·0.25HCl) C, H, N.

Example 5: Preparation of 2-[[3-(chloromethyl)-6-nitroindolin-1-yl]carbonyl]-*N*-[2-(4-morpholinyl)ethyl]indole-6-carboxamide hydrochloride.

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2-(Methoxycarbonyl)indole-6-carboxylic acid was coupled with 4-(2-aminoethyl)-morpholine by the method described above to give 2-(methoxycarbonyl)-N-[2-(4-morpholinyl)ethyl]indole-6-carboxamide as a white powder (78%), mp 226-227.5 °C (MeOH). ¹H NMR [(CD₃)₂SO] δ 12.24 (s, 1 H, indole NH), 8.42 (t, J = 5.6 Hz, 1 H, amide NH), 7.97 (s, 1 H, H-3 or H-7), 7.71 (d, J = 8.4 Hz, 1 H, H-4), 7.56 (dd, J = 8.4, 1.5 Hz, 1 H, H-5), 7.20 (s, 1 H, H-3 or H-7), 3.90 (s, 3 H, CO₂Me), 3.58 (t, J = 4.6 Hz, 4 H, CH₂O), 3.41 (q, J = 6.5 Hz, 2 H, CONHCH₂), 2.48 (t, J = 7.0 Hz, 2 H, CONHCH₂CH₂), 2.42 (t, J = 4.1 Hz, 4 H, NCH₂CH₂O); ¹³C NMR δ 166.6 (CONH), 161.5 (CO₂Me), 136.7, 131.0, 129.1, 128.5 (C-2,6,8,9), 121.6, 118.9, 112.3, 107.5 (C-3,4,5,7), 66.2 (CH₂O), 57.3, 53.2 (CH₂N), 51.9 (OCH₃), 36.6 (NHCH₂). Anal. (C₁₇H₂₁N₃O₄) C, H, N.

2-(Methoxycarbonyl)-N-[2-(4-morpholinyl)ethyl]indole-6-carboxamide was hydrolysed by the method described above to give 2-carboxy-N-[2-(4-morpholinyl)ethyl]indole-6-carboxamide as a white solid (83%), mp 227-233 °C. ¹H NMR [(CD₃)₂SO] δ 12.03 (s, 1 H, indole NH), 8.42 (t, J = 5.6 Hz, 1 H, amide NH), 7.96 (s, 1 H, H-3 or H-7), 7.68 (d, J = 8.4 Hz, 1 H, H-4), 7.54 (dd, J = 8.4, 1.3 Hz, 1 H, H-5), 7.10 (d, J = 1.3 Hz, 1 H, H-3 or H-7), 3.60 (t, J = 4.6 Hz, 4 H, CH₂O), 3.42 (q, J = 6.5 Hz, 2 H, CONHCH₂), 2.53 (t, J = 6.9 Hz, 2 H, CONHCH₂CH₂), 2.50-2.45 (m, 4 H, NCH₂CH₂O); ¹³C NMR δ 166.8 (CONH), 162.7 (CO₂H), 136.5, 131.1, 130.4, 128.7 (C-2,6,8,9), 121.4, 118.6, 112.2, 106.8 (C-3,4,5,7), 66.0 (CH₂O), 57.3, 53.1 (CH₂N), 36.4 (NHCH₂). HRMS (DEI) Calc. for C₁₆H₁₉N₃O₄ 317.13756. Found 317.13693.

1-(t-Butoxycarbonyl)-3-(chloromethyl)-6-nitroindoline was coupled with 2-carboxy-N-[2-(4-morpholinyl)ethyl]indole-6-carboxamide by the method described above and triturated with hot MeOH to give 2-[[3-(chloromethyl)-6-nitroindolin-1-yl]carbonyl]-N-[2-(4-morpholinyl)ethyl]indole-6-carboxamide as a yellow solid (50%), mp 227-228 °C (dec.). ¹H NMR [(CD₃)₂SO] δ 12.15 (s, 1 H, indole NH), 8.98 (d, J = 2.0 Hz, 1 H, H-7), 8.41 (t, J = 5.5 Hz, 1 H, amide NH), 8.04 (dd, J = 8.3, 2.2 Hz, 1 H, H-5), 8.01 (s, 1 H, H-3' or H-7'), 7.76 (d, J = 8.2 Hz, 1 H, H-4 or H-4'), 7.74 (d, J = 8.0 Hz, 1 H, H-4 or H-4'), 7.57 (dd, J = 8.5, 1.1 Hz, 1 H, H-5'), 7.28 (d, J = 1.4 Hz, 1 H, H-3' or H-7'), 4.86 (t, J = 10.1

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Hz, 1 H, H-2), 4.51 (dd, $J = 10.7, 5.2$ Hz, 1 H, H-2), 4.19-4.06 (m, 3 H, H-3 and CH_2Cl), 3.59 (t, $J = 4.5$ Hz, 4 H, CH_2O), 3.42 (q, $J = 6.5$ Hz, 2 H, CONHCH_2), 2.50 (t, $J = 6.9$ Hz, 2 H, $\text{CONHCH}_2\text{CH}_2$), 2.46-2.41 (m, 4 H, $\text{NCH}_2\text{CH}_2\text{O}$); ^{13}C NMR δ 166.7 (CONH), 160.4 (CON), 147.6, 144.6, 144.0, 135.7, 131.8, 130.8, 129.0 (C-6,8,9,2',6',8',9'), 125.5, 121.6, 119.5, 118.9, 112.0, 111.2, 106.1 (C-4,5,7,3',4',5',7'), 66.2 (CH_2O), 57.4 ($\text{CONHCH}_2\text{CH}_2$), 54.0 (C-2), 53.3 ($\text{NCH}_2\text{CH}_2\text{O}$), 47.0 (CH_2Cl), 42.0 (C-3), 36.6 (CONHCH_2). A sample was converted to the hydrochloride salt and triturated with hot EtOH to give 2-[[3-(chloromethyl)-6-nitroindolin-1-yl]carbonyl]-N-[2-(4-morpholinyl)ethyl]indole-6-carboxamide hydrochloride as a pale yellow powder, mp 280-285 °C (dec.). Anal. ($\text{C}_{25}\text{H}_{26}\text{ClN}_5\text{O}_5 \cdot \text{HCl} \cdot \text{H}_2\text{O}$) C, H, N.

Example 6: Preparation of 2-[[3-(chloromethyl)-6-nitroindolin-1-yl]carbonyl]-N-(1,3-dihydroxy-2-propyl)indole-6-carboxamide.

2-(Methoxycarbonyl)indole-6-carboxylic acid was coupled with 2-amino-1,3-bis(t-butyldimethylsilyl)-1,3-propanediol by the method described above and the product purified by dry column chromatography (eluting with 1:4 EtOAc:petroleum ether) to give N-[1,3-bis(t-butyldimethylsilyloxy)-2-propyl]-2-(methoxycarbonyl)indole-6-carboxamide as a pale yellow foam (87%). Trituration with hot petroleum ether gave white needles, mp 127-129 °C. ^1H NMR (CDCl_3) δ 9.24 (s, 1 H, indole NH), 8.03 (s, 1 H, H-3 or H-7), 7.72 (d, $J = 8.3$ Hz, 1 H, H-4), 7.43 (dd, $J = 8.3, 1.5$ Hz, 1 H, H-5), 7.24 (dd, $J = 1.9, 0.8$ Hz, 1 H, H-3 or H-7), 6.64 (d, $J = 8.3$ Hz, 1 H, amide NH), 4.26-4.18 (m, 1 H, CONHCH), 3.97 (s, 3 H, CO_2Me), 3.90 (dd, $J = 9.6, 3.5$ Hz, 2 H, CH_2O), 3.67 (dd, $J = 9.6, 6.4$ Hz, 2 H, CH_2O), 0.92 (s, 18 H, t-Bu), 0.10 (s, 6 H, SiMe), 0.09 (s, 6 H, SiMe); ^{13}C NMR δ 167.0 (CONH), 162.0 (CO_2Me), 136.4, 131.5, 129.6, 129.4 (C-2,6,8,9), 122.6, 118.5, 112.1, 108.5 (C-3,4,5,7), 60.4 (CH_2O), 52.2, 51.9 (CONHCH , OCH_3), 25.9 (t-Bu), 18.2 (SiCMe_3), -5.4, -5.5 (SiMe_2). Anal. ($\text{C}_{26}\text{H}_{44}\text{N}_2\text{O}_5\text{Si}_2$) (*CRL 7726 coming).

A solution of N-[1,3-bis(t-butyldimethylsilyloxy)-2-propyl]-2-(methoxycarbonyl)indole-6-carboxamide (744 mg, 1.43 mmol) and tetrabutylammonium fluoride (2.9 mL of a 1M solution in THF, 2.9

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mmol) in THF (20 mL) was stirred at 20 °C for 80 min and evaporated. The residue was crystallised from MeOH (10 mL) giving *N*-(1,3-dihydroxy-2-propyl)-2-(methoxycarbonyl)indole-6-carboxamide as a white solid (305 mg, 73%), mp 205-207 °C. Dry column chromatography of the mother liquor (eluting with 20:1 EtOAc:MeOH) gave a second crop (57 mg, 14%). ¹H NMR [(CD₃)₂SO] δ 12.22 (s, 1 H, indole NH), 7.97 (s, 1 H, H-3 or H-7), 7.94 (d, J = 8.0 Hz, 1 H, amide NH), 7.70 (d, J = 8.5 Hz, 1 H, H-4), 7.59 (dd, J = 8.5, 1.3 Hz, 1 H, H-5), 7.20 (d, J = 1.6 Hz, 1 H, H-3 or H-7), 4.66 (t, J = 5.7 Hz, 2 H, OH), 4.03-3.95 (m, 1 H, CONHCH), 3.90 (s, 3 H, CO₂Me), 3.54 (t, J = 5.8 Hz, 4 H, CH₂OH). Anal. (C₁₄H₁₆N₂O₅) C, H, N.

N-(1,3-Dihydroxy-2-propyl)-2-(methoxycarbonyl)indole-6-carboxamide was hydrolysed by the method described above to give 2-carboxy-*N*-(1,3-dihydroxy-2-propyl)indole-6-carboxamide as a white solid (97%), mp 240-245 °C. ¹H NMR [(CD₃)₂SO] δ 13.11 (br s, 1 H, CO₂H), 12.05 (s, 1 H, indole NH), 7.98 (s, 1 H, H-3 or H-7), 7.92 (d, J = 7.9 Hz, 1 H, amide NH), 7.70 (d, J = 8.5 Hz, 1 H, H-4), 7.58 (d, J = 8.5 Hz, 1 H, H-5), 7.14 (s, 1 H, H-3 or H-7), 4.4 (br s, 2 H, OH), 4.08-3.92 (m, 1 H, CONHCH), 3.56 (d, J = 5.7 Hz, 4 H, CH₂OH); ¹³C NMR δ 166.8 (CONH), 162.5 (CO₂H), 136.5, 130.8, 130.5, 128.6 (C-2,6,8,9), 121.3, 118.9, 112.4, 107.0 (C-3,4,5,7), 60.4 (CH₂OH), 53.8 (CONHCH). Anal. (C₁₃H₁₄N₂O₅) (*CRL 7729 coming).

1-(*t*-Butoxycarbonyl)-3-(chloromethyl)-6-nitroindoline was coupled with 2-carboxy-*N*-(1,3-dihydroxy-2-propyl)indole-6-carboxamide by the method described above, the EtOAc layer dried (Na₂SO₄), evaporated, and the residue recrystallised from EtOH to give 2-[[3-(chloromethyl)-6-nitroindolin-1-yl]carbonyl]-*N*-(1,3-dihydroxy-2-propyl)indole-6-carboxamide as a cream powder (29%), mp 230-231 °C (dec.). ¹H NMR [(CD₃)₂SO] δ 12.13 (s, 1 H, indole NH), 8.98 (d, J = 2.1 Hz, 1 H, H-7), 8.05 (dd, J = 8.3, 2.2 Hz, 1 H, H-5), 8.04 (s, 1 H, H-3' or H-7'), 7.95 (d, J = 8.0 Hz, 1 H, amide NH), 7.76 (d, J = 8.5 Hz, 1 H, H-4'), 7.75 (d, J = 8.3 Hz, 1 H, H-4), 7.61 (dd, J = 8.5, 1.4 Hz, 1 H, H-5'), 7.29 (d, J = 1.6 Hz, 1 H, H-3' or H-7'), 4.87 (t, J = 10.1 Hz, 1 H, H-2), 4.70 (t, J = 5.7 Hz, 2 H, OH), 4.51 (dd, J = 10.7, 5.1 Hz, 1 H, H-2), 4.19-4.06 (m, 3 H, H-3 and CH₂Cl), 4.04-3.97 (m, 1 H, CONHCH), 3.55 (t, J = 5.7 Hz, 4 H, CH₂OH); ¹³C NMR δ 166.8 (CONH), 160.4 (CON),

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147.6, 144.6, 139.9, 135.7, 131.8, 131.0, 128.9 (C-6,8,9,2',6',8',9'), 125.5, 121.4, 119.4, 119.1, 112.2, 111.2, 106.1 (C-4,5,7,3',4',5',7'), 60.4 (CH₂OH), 54.0 (C-2), 53.8 (CONHCH), 47.0 (CH₂Cl), 42.0 (C-3). Anal. (C₂₂H₂₁ClN₄O₆).

5 Example 7: Preparation of N-(carboxymethyl)-2-[[3-(chloromethyl)-6-nitroindolin-1-yl]carbonyl]indole-6-carboxamide.

2-(Methoxycarbonyl)indole-6-carboxylic acid was coupled with glycine t-butyl ester hydrochloride in the presence of NaHCO₃ (1 equivalent) by the method described above to give N-
10 [(butoxycarbonyl)methyl]-2-(methoxycarbonyl)indole-6-carboxamide as a white solid (86%), mp 181-183.5 °C (EtOAc). ¹H NMR (CDCl₃) δ 9.58 (s, 1 H, indole NH), 8.06 (s, 1 H, H-3 or H-7), 7.68 (d, J = 8.4 Hz, 1 H, H-4), 7.52 (dd, J = 8.4, 1.5 Hz, 1 H, H-5), 7.20 (dd, J = 2.0, 0.8 Hz, 1 H, H-3 or H-7), 6.82 (t, J = 4.7 Hz, 1 H, amide NH), 4.20 (d, J = 5.0 Hz, 2 H, CH₂), 3.96 (s, 3 H, CO₂Me),
15 1.51 (s, 9 H, t-Bu); ¹³C NMR δ 169.5, 167.7, 162.1 (3 x CO), 136.4, 130.6, 129.7, 129.5 (C-2,6,8,9), 122.5, 118.8, 112.3, 108.4 (C-3,4,5,7), 82.6 (OCMe₃), 52.2 (OCH₃), 42.7 (CONHCH₂), 28.1 (t-Bu). Anal. (C₁₇H₂₀N₂O₅) C, H, N.

20 A mixture of N-[(butoxycarbonyl)methyl]-2-(methoxycarbonyl)indole-6-carboxamide (0.94 g, 2.8 mmol) and LiOH.H₂O (0.19 g, 4.5 mmol) in THF (50 mL) and H₂O (15 mL) was stirred at 20 °C for 15 h then at 65 °C for 7 h, cooled, and the THF evaporated. EtOAc was added, the mixture extracted with aq.
25 NaHCO₃, and the aqueous layer acidified. Extraction with EtOAc gave a mixture containing the required product and the corresponding diacid. Dry column chromatography (eluting with CHCl₃:EtOH 10:1) gave N-[(butoxycarbonyl)methyl]-2-carboxyindole-6-carboxamide as a white powder (97 mg, 11%), mp 267-268.5 °C.
30 ¹H NMR [(CD₃)₂SO] δ 13.15 (br s, 1 H, CO₂H), 12.11 (s, 1 H, indole NH), 8.82 (t, J = 5.8 Hz, 1 H, amide NH), 7.99 (s, 1 H, H-3 or H-7), 7.71 (d, J = 8.5 Hz, 1 H, H-4), 7.56 (dd, J = 8.5, 1.2 Hz, 1 H, H-5), 7.13 (s, 1 H, H-3 or H-7), 3.90 (d, J = 5.8 Hz, 2 H, CH₂), 1.43 (s, 9 H, t-Bu). MS (DEI) m/z 318 (30%, M⁺), 188
35 (100%); HRMS calcd. for C₁₆H₁₆N₂O₅ 318.12157, found 318.12136.

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1-(*t*-Butoxycarbonyl)-3-(chloromethyl)-6-nitroindoline was coupled with *N*-[(butoxycarbonyl)methyl]-2-carboxyindole-6-carboxamide by the method described above and the product recrystallised from EtOAc to give with *N*-[(butoxycarbonyl)methyl]-2-[[3-(chloromethyl)-6-nitroindolin-1-yl]carbonyl]indole-6-carboxamide as a yellow solid (69%), mp 286 °C (dec.). ¹H NMR [(CD₃)₂SO] δ 12.20 (d, *J* = 1.5 Hz, 1 H, indole NH), 8.99 (d, *J* = 2.2 Hz, 1 H, H-7), 8.50 (t, *J* = 6.0 Hz, 1 H, amide NH), 8.06 (s, 1 H, H-3' or H-7'), 8.05 (dd, *J* = 8.2, 2.2 Hz, 1 H, H-5), 7.78 (d, *J* = 8.5 Hz, 1 H, H-4'), 7.75 (d, *J* = 8.2 Hz, 1 H, H-4), 7.60 (dd, *J* = 8.5, 1.4 Hz, 1 H, H-5'), 7.30 (d, *J* = 1.6 Hz, 1 H, H-3' or H-7'), 4.87 (t, *J* = 10.1 Hz, 1 H, H-2), 4.51 (dd, *J* = 10.7, 5.2 Hz, 1 H, H-2), 4.19-4.08 (m, 3 H, H-3 and CH₂Cl), 3.92 (d, *J* = 5.9 Hz, 2 H, CONHCH₂), 1.44 (s, 9 H, *t*-Bu); ¹³C NMR δ 169.1 (CO₂*t*Bu), 167.1 (CONH), 160.4 (CON), 147.6, 144.6, 140.0, 135.7, 132.0, 130.0, 129.2 (C-6,8,9,2',6',8',9'), 125.5, 121.7, 119.4, 118.9, 112.2, 111.2, 106.1 (C-4,5,7,3',4',5',7'), 80.5 (OCMe₃), 54.0 (C-2), 47.0 (CH₂Cl), 42.0 (C-3), 41.9 (CH₂CO₂*t*Bu), 27.7 (*t*-Bu). Anal. (C₂₅H₂₅ClN₄O₆) C, H, N.

A suspension of *N*-[(butoxycarbonyl)methyl]-2-[[3-(chloromethyl)-6-nitroindolin-1-yl]carbonyl]indole-6-carboxamide (70 mg, 0.14 mmol) in HCl-saturated dioxane (20 mL) was stirred at 20 °C for 2 h, most of the dioxane evaporated, and the residue triturated with hot EtOAc to give *N*-(carboxymethyl)-2-[[3-(chloromethyl)-6-nitroindolin-1-yl]carbonyl]indole-6-carboxamide as a pale yellow powder (50 mg, 80%), mp 281 °C (dec.). ¹H NMR [(CD₃)₂SO] δ 12.57 (br s, 1 H, CO₂H), 12.20 (d, *J* = 1.3 Hz, 1 H, indole NH), 8.99 (d, *J* = 2.2 Hz, 1 H, H-7), 8.84 (t, *J* = 5.9 Hz, 1 H, amide NH), 8.07 (s, 1 H, H-3' or H-7'), 8.05 (dd, *J* = 8.3, 2.2 Hz, 1 H, H-5), 7.79 (d, *J* = 8.4 Hz, 1 H, H-4'), 7.75 (d, *J* = 8.3 Hz, 1 H, H-4), 7.61 (dd, *J* = 8.4, 1.3 Hz, 1 H, H-5'), 7.30 (d, *J* = 1.9 Hz, 1 H, H-3' or H-7'), 4.87 (t, *J* = 10.1 Hz, 1 H, H-2), 4.51 (dd, *J* = 10.7, 5.2 Hz, 1 H, H-2), 4.19-4.08 (m, 3 H, H-3 and CH₂Cl), 3.95 (d, *J* = 5.8 Hz, 2 H, CONHCH₂). Anal. (C₂₁H₁₇ClN₄O₆) C, H, N.

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Example 8: Preparation of dimethyl 2-(S, R)-[N-[3-(S,R)-(chloromethyl)-1-[(5',6',7'-trimethoxyindol-2'-yl)carbonyl]indolin-6-yl]ureylene]pentanedicarboxylic acid.

A solution of dimethyl (S)-2-isocyanatopentanedioate (116 mg, 0.58 mmol) [prepared by the method of J.S. Nowick, N.A. Powell, T.M. Nguyen and G. Noronha, *J. Org. Chem.*, (1992), 57, 7364] in dry THF (1 mL) was added to a stirred solution of 6-amino-3-(chloromethyl)-1-[(5',6',7'-trimethoxyindol-2'-yl)carbonyl]indoline (228 mg, 0.55 mmol) in dry THF (8 mL) at 20 °C. TLC analysis after 40 min indicated incomplete reaction. More isocyanate (0.33 g, 1.6 mmol) in THF (1 mL) was added, and after a further 20 min the reaction was quenched by the addition of H₂O (10 mL) and stirred overnight. The THF was evaporated, the aqueous residue extracted with EtOAc, and the organic layer dried (Na₂SO₄) and evaporated. Column chromatography (eluting with 3:1 EtOAc:petroleum ether) gave a mixture (¹H NMR) of the urea derived from (S)-glutamic acid dimethyl ester and the desired dimethyl 2-(S)-[N-[3-(S,R)-(chloromethyl)-1-[(5',6',7'-trimethoxyindol-2'-yl)carbonyl]indolin-6-yl]ureylene]pentanedioate (ca. 280 mg, ca. 80%) as a pale yellow solid.

This mixture was dissolved in MeOH (20 mL), a solution of cesium carbonate (3.3 g, ca. 10 mmol) in H₂O (5 mL) added, and the yellow solution stirred at 20 °C for 24 h. The mixture was washed with EtOAc, the aqueous layer acidified (2N HCl), extracted with EtOAc (x2), and the extracts dried (Na₂SO₄) and evaporated. Crystallisation from EtOAc-MeOH gave 2-(S, R)-[N-[3-(S,R)-(chloromethyl)-1-[(5',6',7'-trimethoxyindol-2'-yl)carbonyl]indolin-6-yl]ureylene]pentanedicarboxylic acid as an off-white powder (108 mg, 33% for two steps), mp 173-174 °C (dec.). ¹H NMR [(CD₃)₂SO] δ 12.48 (br s, 2 H, CO₂H), 11.42 (d, J = 1.5 Hz, 1 H, indole NH), 8.75 (s, 1 H, NH), 8.13 (s, 1 H, H-7), 7.28 (s, 2 H, H-4,5), 7.01 (d, J = 2.2 Hz, 1 H, H-3'), 6.96 (s, 1 H, H-4'), 6.40 (d, J = 8.0 Hz, 1 H, NH), 4.61 (dd, J = 10.9, 8.4 Hz, 1 H, H-2), 4.26 (dd, J = 10.9, 4.4 Hz, 1 H, H-2), 4.26-4.18 (m, 1 H, CHCO₂H), 4.01-3.94 (m, 1 H, CHCH₂Cl), 3.93 (s, 3 H, OCH₃), 3.84-3.78 (m, 2 H, CHCH₂Cl), 3.82 (s, 3 H, OCH₃), 3.79 (s, 3 H, OCH₃), 2.38-2.22 (m, 2 H, CH₂CH₂CO₂H), 2.06-1.97 (m, 1 H,

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CH₂CH₂CO₂H), 1.86-1.76 (m, 1 H, CH₂CH₂CO₂H); ¹³C NMR δ 173.9, 173.6 (2 x CO₂H), 160.1 (amide carbonyl), 154.7, 149.1, 143.9, 140.2, 139.8, 139.0, 130.9, 125.2, 124.7, 123.1 (urea carbonyl, C-6,8,9,2',5',6',7',8',9'), 124.5, 113.1, 106.8, 105.9, 98.0 (C-4,5,7,3',4'), 61.0, 60.9, 55.9 (3 x OCH₃), 54.3 (C-2), 51.5 (CHCO₂H), 47.7 (CH₂Cl), 41.8 (C-3), 29.9, 27.2 (CH₂CH₂CO₂H). Anal. (C₂₇H₂₉ClN₄O₉·0.5H₂O) C,H,N.

Example 9: Biological activity.

The compounds of Examples 1 and 3 show potent cytotoxicity to mammalian tumour cells, and are thus of interest as anticancer drugs. The free amine (compound of Example 1) shows potent cytotoxicity to mammalian tumour cells, and is thus of interest as an anticancer drug. It has an IC₅₀ in AA8 cells of 0.32 μM, and against UV4 cells of 0.059 μM. The corresponding prodrug (of Example 3) is expectedly much less cytotoxic (an IC₅₀ in AA8 cells of 23 μM and against UV4 cells of 19.6 μM). For these evaluations, AA8 and UV4 cells were maintained in exponential phase growth (doubling times 14 and 15 h respectively) using Alpha MEM containing fetal calf serum (10% v/v) without antibiotics, and were subcultured twice weekly by trypsinization. Bulk cultures were prepared for experiments by seeding cells in spinner flasks at 10⁴ cells/mL in the above medium with addition of penicillin (100 IU/mL) and streptomycin (100 μg/mL). Cultures were initiated in 96-well microtiter trays to give 200 (AA8) or 300 (UV4) cells in 0.05 mL per well. After growth in a CO₂ incubator for 24h, drugs were added in culture medium, using serial two-fold dilutions to provide duplicate cultures at five different concentrations for each of eight drugs (plus eight controls) per tray. After 18h drugs were removed by washing cultures three times with fresh medium, and the trays were incubated for a further 78h. Cell density was then determined by staining with methylene blue as described [Finlay, G.J.; Baguley, B.C; Wilson, W.R. Anal. Biochem., 1984, 139, 272-277]. The IC₅₀ was calculated as the drug concentration providing 50% inhibition of growth relative to the controls.

Example 10: Activation of Prodrug by nitroreductase.

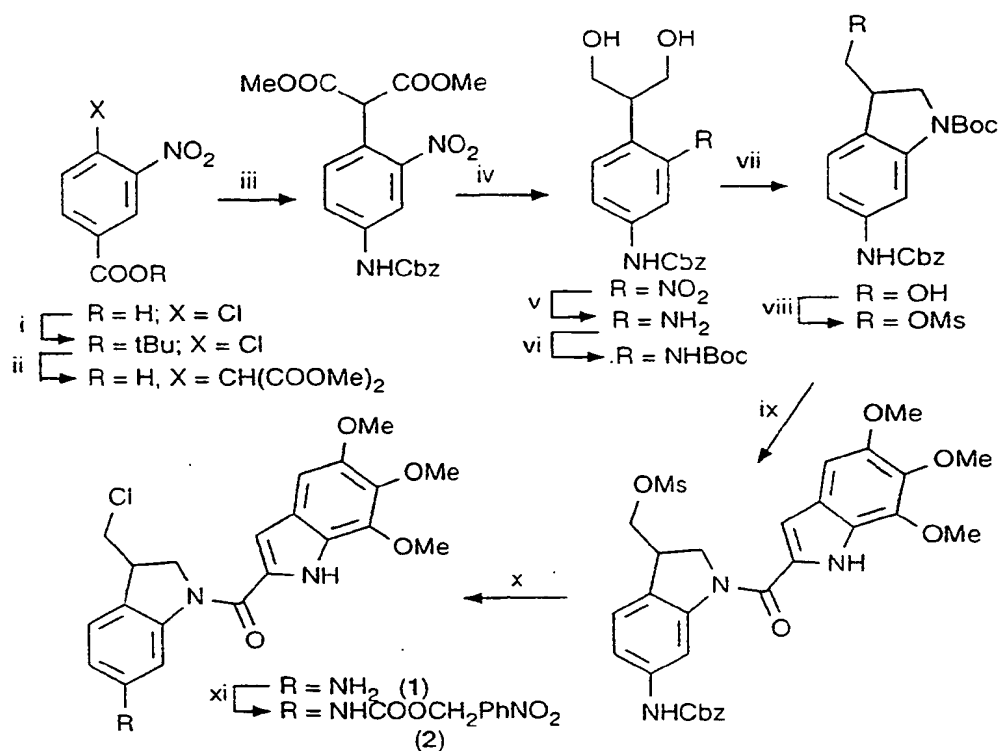
The compound of the formula (II) produced in example 3 also shows high levels of activation by the isolated *E. coli* NR2 nitroreductase enzyme, as shown by the following experiment.

- 5 The compound was incubated with UV4 cells for 18 hours in 96-well plates under aerobic conditions. The UV4 cell line is a mutant Chinese hamster ovary fibroblast lines derived from the wild-type AA8. It is a UV complementation group 1 mutant, with a defect in the incision step of excision repair [Thompson, L.H. et al Proc. Natl. Acad. Sci (USA), 1980, 78, 3734; Thompson, L.H.; Carrono, A.V. In Cellular responses to DNA Damage, UCLA Symposium on Molecular and Cellular Biology, New Series, Vol. 11 (Freidberg, E.C.; Bridges, B.R.; Eds), Alan R. Liss: New York, 1983: p 125], and is highly sensitive to agents which form bulky DNA monoadducts or crosslinks [Hoy, C.A. et al Cancer Res., 1985, 45, 1737-1747].
10 The IC_{50} of the compound was determined as described above in Example 9 and found to be 11.9 μM .
15

- The experiment was repeated but in addition purified *E. coli* nitroreductase enzyme (1 $\mu g/mL$) and NADH (1 mM, as cofactor) was
20 added during the entire time of the incubation. The IC_{50} was determined and found to be 0.46 μM . This represents a 26-fold activation by the enzyme.

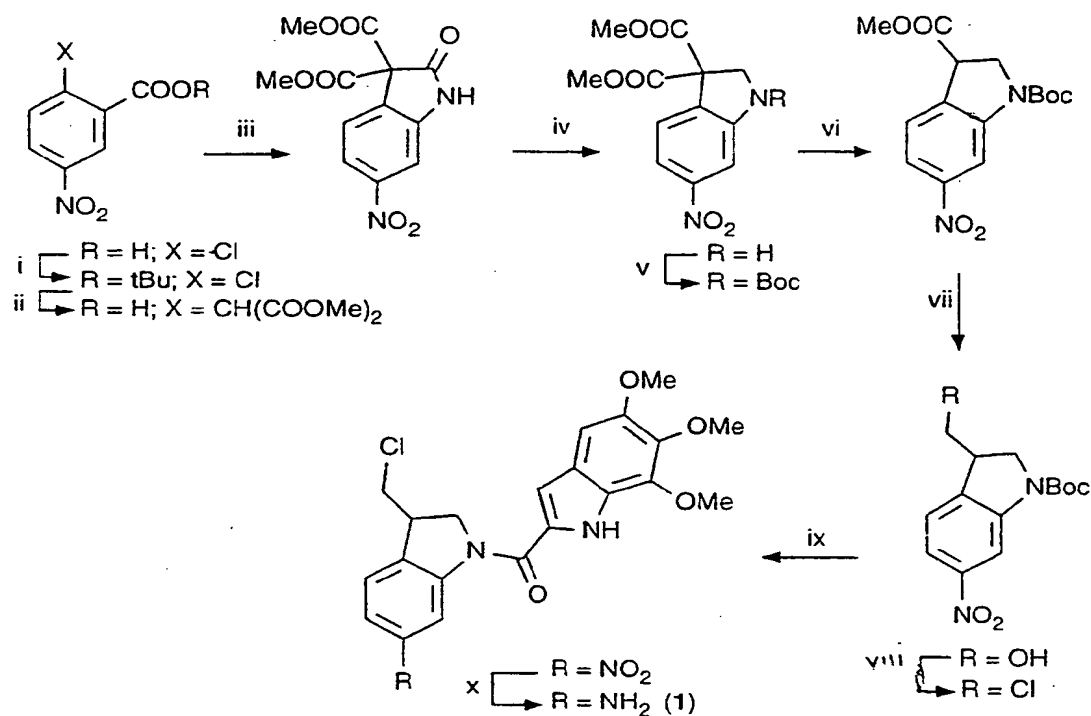
- A similar set of experiments on the compound of formula (I) produced in example 1(h) also showed significant activation (IC_{50} 2.34 μM in the absence of *E. coli* nitroreductase, IC_{50} 0.006 μM in the presence of *E. coli* nitroreductase, representing a 400-fold
25 activation by the enzyme.

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Scheme 1

- i $SOCl_2$, then $KOtBu$
- ii $NaH/CH_2(COOMe)_2$, then $HCOOH$
- iii $SOCl_2$, then NaN_3 , then $PhCH_3/heat/PhCH_2OH$
- iv iBu_2AlH
- v PtO_2/H_2
- vi $(Boc)_2O/Na_2CO_3$
- vii $DEAD/PPh_3$
- viii $MsCl/Et_3N$
- ix HCl , then $EDCI/5,6,7-triOMeindole-2-carboxylic acid$
- x $Pd-C/H_2/NH_4^+HCO_2^-$, then $LiCl$
- xi $4-NO_2PhCH_2OCOCl$



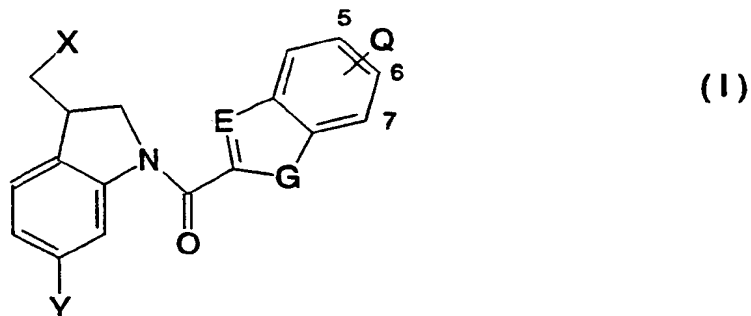
Scheme 2

- i $SOCl_2$ then $KOtBu$
 ii $NaH/CH_2(COOMe)_2$ then $HCOOH$
 iii $(PhO)_2PON_3/Et_3N$
 iv $BH_3.DMS$
 v $(Boc)_2O/DMAP$
 vi $NaOMe$ then CF_3CO_2H
 vii iBu_2AlH
 viii $MsCl/Et_3N$ then $LiCl$
 ix HCl then $EDCI/5,6,7\text{-triOMeindole-2-carboxylic acid}$
 x PtO_2/H_2

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CLAIMS

1. A compound of the formula (I):



wherein:

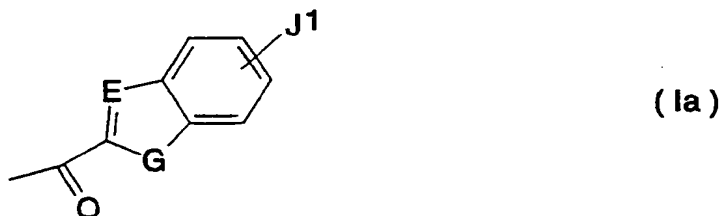
X is halogen or OSO_2R , where R represents hydrogen or lower straight or branched alkyl (up to five carbon atoms) optionally substituted with from 1 to 4 hydroxyl, acid (COOH) or amino groups which amino groups are optionally substituted by one or two lower alkyl groups;

Y is NH_2 , NO_2 , NHOH , NHR , NRR , N(O)RR , NROH , SR or SSR , where R is defined as above, but that in the case where Y is SSR , then R can also be another moiety of formula (I) (i.e., a symmetrical disulfide);

E is $-\text{N}=\text{}$ or $-\text{CH}=\text{}$;

G is O, S or NH; and

Q is from one to three of H, OR, NRR , CONHR , NHCOR or NHCONHR at any one of positions 5 to 7 where R is defined as above (which may be the same or different when Q is two or three), a group of formula (Ia):

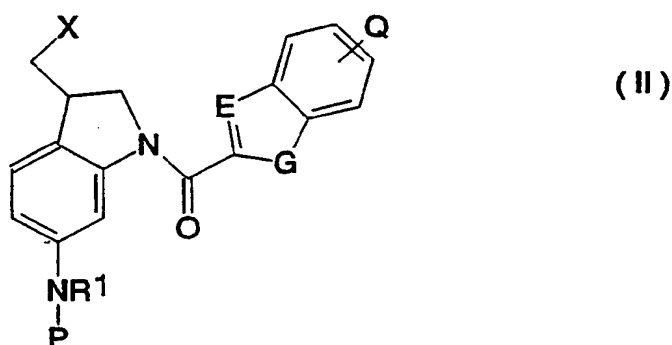


where E and G are as defined above, J^1 is up to three groups, H, OR or NRR (which may be the same or different when J^1 is two or

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three) where R is as defined above, or is a group of the formula -CONHJ², -NHJ² or -OJ² where J² is a group -(CH₂)_mHt where m is an integer from 1 to 4 and Ht is a carbon or heterocyclic ring containing 5 or 6 atoms, one or two of which may be oxygen, sulphur or nitrogen (the remainder being carbon);
or a physiologically functional derivative thereof.

2. A compound of the formula (II):



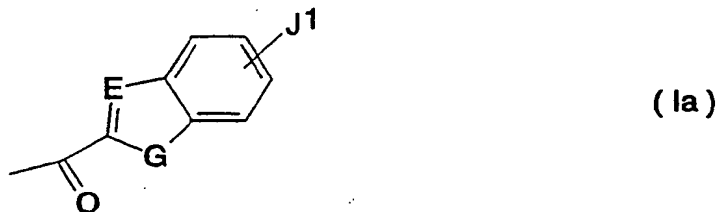
wherein:

X is halogen or OSO₂R, where R represents hydrogen, lower straight or branched alkyl (up to five carbon atoms) optionally substituted with from 1 to 4 hydroxyl, acid (COOH) or amino groups which amino groups are optionally substituted by one or two lower alkyl groups;

E is -N= or -CH=;

G is O, S or NH; and

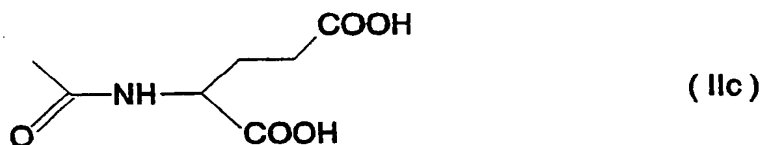
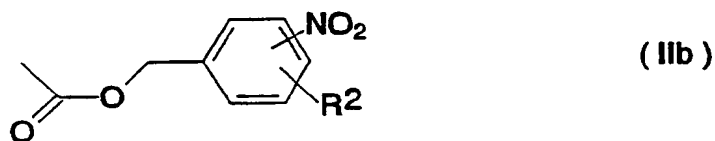
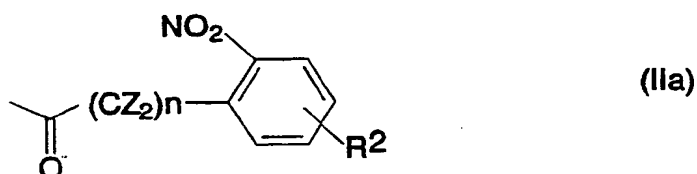
Q is from one to three of H, OR, NRR, CONHR, NHCOR or NHCONHR at any one of positions 5 to 7 where R is defined as above (which may be the same or different when Q is two or three), a group of formula (Ia):



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where E and G are as defined above, J^1 is up to three groups, H, OR or NRR (which may be the same or different when J^1 is two or three) where R is as defined above, or is a group of the formula -CONH J^2 , -NH J^2 or -O J^2 where J^2 is a group -(CH₂)_mHt where m is an integer from 1 to 4 and Ht is a carbon or heterocyclic ring containing 5 or 6 atoms, one or two of which may be oxygen, sulphur or nitrogen (the remainder being carbon);

R^1 is a group R where R is as defined above, and P is selected from the structures of formulae (IIa, IIb or IIc):



wherein:

Z is H or Me;

n is 1 or 2; and

R^2 is a group R, CONHR, NHCOR, OR or SO₂R, where R is as defined above;

or a physiologically functional derivative thereof.

3. A compound according to claim 1 or 2 wherein X is chloro.

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4. A compound according to any one of claims 1 to 3 wherein Q represent three methoxy groups at positions 5, 6 and 7 of the indol grouping.
5. 6-Amino-3-(chloromethyl)-1-[(5',6',7'-trimethoxyindol-2'-yl)carbonyl]indoline or a physiologically functional derivative thereof.
6. 3-(Chloromethyl)-6-[(4''-nitrobenzyloxycarbonyl)amino]-1-[(5',6',7'-trimethoxyindol-2'-yl)carbonyl]indoline or a physiologically functional derivative thereof.
7. 2-[[3-(chloromethyl)-6-nitroindolin-1-yl]carbonyl]-N-[2-(2-pyridinyl)ethyl]indole-6-carboxamide or a physiologically functional salt thereof.
8. 2-[[3-(chloromethyl)-6-nitroindolin-1-yl]carbonyl]-N-[2-(4-morpholinyl)ethyl]indole-6-carboxamide or a physiologically functional salt thereof.
9. 2-[[3-(chloromethyl)-6-nitroindolin-1-yl]carbonyl]-N-(1,3-dihydroxy-2-propyl)indole-6-carboxamide.
10. N-(carboxymethyl)-2-[[3-(chloromethyl)-6-nitroindolin-1-yl]carbonyl]indole-6-carboxamide
11. A two component system for the treatment of neoplastic disease which comprises:
 - (i) a vector encoding and capable of expressing a nitroreductase enzyme in a tumour cell; and
 - (ii) a compound according to any one of claims 1 to 10.
12. A two component system for the treatment of neoplastic disease which comprises:
 - (i) a tumour-directed antibody linked to a nitroreductase enzyme; and
 - (ii) a compound according to any one of claims 1 to 10.

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13. A composition comprising a compound according to any one of claims 1 to 10 together with a pharmaceutically acceptable carrier or diluent.
14. A compound according to any one of claims 1 to 10, a system according to claims 11 or 12, or a composition according to claim 13 for use in a method of treatment of the human or animal body.
15. A method of treating neoplastic disease which comprises administering to a patient in need of treatment an effective amount of a compound according to any one of claims 1 to 10, a system according to claims 11 or 12, or a composition according to claim 13.

INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/NZ 96/00083

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D209/42 A61K31/40 C07D401/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|---|-----------------------|
| A | WO 91 16324 A (THE UPJOHN COMPANY) 31 October 1991 cited in the application see claims | 1 |
| A | WO 88 04659 A (THE UPJOHN COMPANY) 30 June 1988 cited in the application see claims | 1 |
| A | JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, vol. 112, no. 24, - 1990 DC US, pages 8961-8971, XP002022071 DALE L. BOGER ET AL.: "Duocarmycin-pyrindamycin DNA alkylation properties and ..." see page 8966 - page 8967 | 1 |

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

2 January 1997

Date of mailing of the international search report

13.01.97

Name and mailing address of the ISA

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Authorized officer

Van Bijlen, H

INTERNATIONAL SEARCH REPORT

International Application No

PCT/NZ 96/00083

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|---|-----------------------|
| A | JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, vol. 112, no. 13, - 1990 DC US, pages 5230-5240, XP002022072 DALE L. BOGER ET AL: "Synthesis of N-(phenylsulfonyl)-9-Cl, ..." see page 5233 - page 5234 --- | 1 |
| A | WO 88 07378 A (CANCER RESEARCH CAMPAIGN TECHNOLOGY LTD.) 6 October 1988 cited in the application see claims ----- | 11 |

INTERNATIONAL SEARCH REPORT

International application No.

PCT/NZ 96/ 00083

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claim 15 is directed to a method of treatment of (diagnostic method practised on) the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/NZ 96/00083

| Patent document cited in search report | Publication date | Patent family member(s) | | Publication date |
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| | | AU-A- | 7795491 | 11-11-91 |
| | | CA-A- | 2078118 | 26-10-91 |
| | | EP-A- | 0527189 | 17-02-93 |
| | | JP-T- | 5508394 | 25-11-93 |
| ----- | | | | |
| WO-A-8804659 | 30-06-88 | AT-T- | 112279 | 15-10-94 |
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| | | DE-T- | 3750612 | 02-03-95 |
| | | EP-A- | 0340243 | 08-11-89 |
| | | KR-B- | 9609264 | 16-07-96 |
| | | US-A- | 5332837 | 26-07-94 |
| ----- | | | | |
| WO-A-8807378 | 06-10-88 | DE-D- | 3889340 | 01-06-94 |
| | | DE-T- | 3889340 | 01-09-94 |
| | | EP-A- | 0408546 | 23-01-91 |
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